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An Investigation of the Bioactivity of Irish Seaweeds and Potential Applications as Nutraceuticals.

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An Investigation of the Bioactivity of Irish Seaweeds and Potential Applications as Nutraceuticals

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A thesis submitted to the Dublin Institute of Technology, in
accordance with the requirements for the degree of

Doctor of Philosophy

**School of Food Science and Environmental Health
College of Sciences & Health**

Supervised by:

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Health, Dublin Institute of Technology

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Abstract

The primary goals of this research were to; identify the bioactivity of a range of edible Irish seaweeds, to examine the current technological procedures currently applied with respect to seaweed processing and in light of this information, to examine the potential of incorporating seaweeds into traditional food products in order to enhance their nutraceutical properties. Currently most Irish seaweeds are washed, dried and packaged, with little done to increase consumer appeal or interest and dried seaweed may appear inaccessible and unfamiliar to consumers. Seaweeds possess an excellent nutritional quality, such as high fibre and phytochemical content, low cholesterol and low glycaemic index (G.I.) and therefore have the potential for classification as functional foods. The first stage of the research was to assess the bioactivity of a range of edible Irish seaweeds, including brown, red and green species, which were available throughout the year (*Laminaria digitata*, *Laminaria saccharina*, *Himanthalia elongata*, *Palmaria palmata*, *Chondrus crispus* and *Enteromorpha spirulina*.) Brown seaweed, *H. elongata*, contained the highest levels of bioactivity in terms of antioxidants and antimicrobials, and was therefore the primary seaweed focused on for the remainder of the research. As seaweeds require some processing to make them edible and palatable, optimal processing treatment was established by investigating the effect of a range of common thermal processing methods such as; drying, boiling, steaming and microwaving on the phytochemical content. It was found that a drying pre-treatment before hydrothermal processing reduced the time required for processing (15 min less) and subsequent phytochemical losses (9% reduction). Atmospheric drying is the most common preservation method in Ireland for seaweed yet there is little effort made to optimise the process in order to capitalise on the nutritional content. With these facts in mind,

drying kinetics under controlled conditions were investigated and mathematically modelled. In terms of phytochemical content, drying was optimised at 40 °C for 2 hours. Under these conditions, reducing the moisture content by 50% caused an increase in phenolic content up to 41%. Rehydration kinetics were also investigated using Response Surface Methodology (RSM) and phytochemical content was maximised by rehydrating at 80.5 °C for 20.4 min. The processed seaweeds were successfully incorporated into commonly consumed convenience products (breadsticks and beef-patties) in order to increase phytochemical and fibre levels and the products were sensorially acceptable as determined by a sensory panel. The overall results of this study indicated that reductions in bioactivity due to application of the current technological procedures could be minimised under controlled conditions. The seaweeds processed with optimal phytochemical levels were successfully incorporated into convenience products with enhanced functionality. Such foods would provide innovative new products which could support the Irish seaweed industry while promoting seaweed to a market who otherwise would not consume such a food.

Declaration

I hereby certify that this thesis which I now submit for examination for the award of Doctor of Philosophy, is entirely my own work and has not been taken from the work of others save and to the extent such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for postgraduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the Institutes guidelines for ethics in research.

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Signed: _____ Date: _____

Candidate

*To my mother, Breda Cox
For everything over the past 30 years,
This is for you.*

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Abbreviations list

µg	Microgram
µl	Microlitre
a*	Degree of red (+a*) to green (-a*)
a, c, k	Parameters in the dehydration models
AlCl ₃	Aluminium chloride
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AscA	Ascorbic acid
b*	Degree of yellow (+b*) to blue (-b*)
BC	Before Christ
BHA	Butylated hydroxyanisole
BHT	Butlyated hydroxytoluene
BIM	Bord Iscaigh Mhara
<i>C</i>	Quality factor
CE	Catechin equivalent
CFU	Colony forming units
CIE	International Commission of Illumination
cm	Centimetre
db	Dry basis
dd	Double distilled
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
Ea	Activation energy for moisture diffusion (kJ/mol)
EC ₅₀	Efficient Concentration that causes 50% activity loss
EU	European Union

FAO	Food and Agricultural Organisation
FDA	Food and Drugs Administration
Fig.	Figure
<i>F</i> -value	Fisher test value
fw	Fresh weight
g	gram
GAE	Gallic acid equivalent
h	Hour
ha	Hectares
HCl	Hydrochloric acid
J	Joule
k	Number of factors in RSM
<i>k</i>	Rate constant
kV	Kilovolts
L	Litre
L*	Lightness index
m	Metre
M	Molar
mbar	Millibar
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
mol	Mole
MR	Moisture ratio

N	Newtons
NaOH	Sodium hydroxide
nm	Nanometres
P	Probability
PCA	Plate count agar
ppm	Parts per million
QE	Quercetin equivalent
R^2	Co-efficient of determination
RMSE	Root mean square error
rpm	Revolutions per minute
RR	Rehydration ratio
RSM	Response surface methodology
s	Second
SCFA	Short-chain fatty acids
SD	Standard deviation
Spp.	Species
SS	Sum square error
T	Temperature (Kelvin or °C)
t	Time (h)
TDF	Total dietary fibre
TFC	Total flavonoid content
TPC	Total phenolic content
TTC	Total condensed tannin content
US	United States
USA	Unites States of America

UV	Ultraviolet
W	Moisture content at any time (g H ₂ O/ g dry basis)
W_e	Equilibrium moisture content (g H ₂ O/ g dry basis)
W_o	Initial moisture content (g H ₂ O/g dry basis)
$X(t)$	Instantaneous moisture content (kg H ₂ O/kg dry matter)
X_0	Initial moisture content (kg H ₂ O/kg dry matter)
α	Alpha
$\alpha, \beta, k_1, k_2, k_{R1}$ and k_{R2}	Parameters in the rehydration models
β	Beta
χ^2	Chi square

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CHAPTER 1

GENERAL INTRODUCTION

A brief explanation of the motivation behind the work, and a summary of the principal objectives

1.1 Motivation

Due to advances in our understanding of the relationship between diet and health, consumers are increasingly more interested in foods that not only adequately meet nutritional needs but also confer health benefits, hence the growing demand for “functional foods” (Cofrades *et al.*, 2008). The well-known correlation between diet and health demonstrates the great possibilities of food to maintain or even improve our health. This has brought about an interest for seeking new products that can contribute to improving our health and well-being (Plaza *et al.*, 2008). Over the past few decades there have been many changes in our food habits and lifestyles. Diets in the Western countries are often high in calories, saturated fats and sugars and low in dietary fibre. This, together with a decrease in physical activity, has given rise to an increase in obesity problems, heart diseases, diabetes and hypertension within the population (Geslain-Lanéelle, 2006).

Marine algae or seaweeds are still identified as an under-exploited plant resource although they have been used in food diets as well as traditional remedies for centuries (Heo *et al.*, 2009). In recent years, seaweeds have served as important sources of bioactive natural substances with various biological activities and potential health benefits (Pangestuti and Kim, 2011). Marine food, due to its phenomenal biodiversity is a treasure house of novel healthy food ingredients and biologically active compounds (Kadam and Prabhasankar, 2010). Seaweed is a source of biologically active phytochemicals, which include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol and phycocyanins. Many of these compounds have been recognised to possess biological activity and hence beneficial for use in human and animal healthcare. Some of the

potential benefits of seaweed consumption include control of hyperlipidemia, thrombosis, tumor, and obesity (Plaza *et al.*, 2008). Seaweeds are high in fibre and can therefore be exploited in order to enrich the fibre contents of foods which are generally low in this component. The content of total dietary fibre in seaweeds ranges from 33 – 50 g/100 g dry basis (db) (Rupérez and Saura-Calixto, 2001). Such properties of seaweed have the potential to be exploited for the development of new functional foods.

In 2007, the Marine Institute of Ireland initiated *Sea Change: A marine knowledge, research and innovation strategy for Ireland 2007 – 2013*. The strategy was aimed to drive the development of marine resources in Ireland in a manner which contributes to the knowledge economy. Research priorities which were highlighted included the need for extraction of bioactive compounds and the development of functional foods from seaweeds. Since then, the 2011 Bord Iscaigh Mhara (BIM or Irish Fisheries Board) *Market Analysis towards the Further Development of Seaweed Aquaculture in Ireland* report has outlined the need for the seaweed industry to develop processing in order to add value to their products. It was also noted that significant technical development is required in the areas of drying, milling and production of liquid extracts along with the need for a science-based approach to the development and marketing of novel seaweed products including functional foods (Walsh and Watson, 2011).

The seaweed industry was also highlighted as a research interest in 2011 in the Irish Governments initiative to develop an '*Integrated Marine Plan for Ireland*' to harness our 'ocean wealth'. Ireland's seaweed and biotechnology sector is estimated at €18

million per annum. There are 36, 000 tonnes of wild seaweed harvested per year and there are currently 185 full time employees (Morrissey *et al.*, 2011). According to the Sea Change Strategy (2006), the Irish seaweed production and processing sector will be worth €30 million per annum by 2020 which opens up opportunities for new developments such as functional foods.

1.2 Objectives

Motivated by the growing consumer demand for foods which contain functional benefits and the potential to explore a relatively untapped abundant natural resource; the purpose of this work was to review the current processing techniques applied in the Irish seaweed industry and to examine their effects on the bioactivity of seaweed. Once the processing was optimised the objective was to develop new functional foods by incorporating seaweeds into traditional Irish food products. Application of seaweeds into the food chain has been limited for a number of reasons. Harvesting technology is underdeveloped and most seaweeds are handpicked from the wild and significantly affected by weather and seasonality. Culturally, seaweeds have not been widely consumed in Ireland with consumption being limited to rural coastal regions. This could be due to the sensory properties of seaweed such as the texture or aroma or the short shelf life after harvesting which would lead to transportation issues. The organoleptic properties of seaweeds are quite different from terrestrial vegetables which may affect the palatability amongst consumers who are unfamiliar with them. Within the aims of the current research, was to process the seaweed in such a manner to reduce the potency of the aroma along with tenderising the texture to a level which is more palatable to the consumer. Also, incorporation of the seaweeds into existing food products which are commonly consumed would be an interesting form

in which to promote seaweeds as there would be some masking of the sensory properties by the additional ingredients.

Specifically, the research focussed on species of edible seaweeds which were available throughout the year on Irish coastlines. Seaweeds are commonly dried outdoors in tunnels under atmospheric conditions. This process is therefore inconsistent as seasonality and weather conditions vary greatly, particularly in Ireland. The dried seaweeds are then processed further by rehydration in hot water to make them palatable before consumption. The present study first aimed to observe the effects of common food processing methods on the seaweed and to optimise the drying and rehydration procedures before incorporating such optimised seaweeds into food products. Table 1.1 outlines the main tasks of the study, the questions which were explored and the principal investigations involved in the analysis of the phytochemicals in seaweeds, and the effects of processing conditions on such bioactivity, leading up to the development of new functional foods.

Table 1.1 Tasks, questions and principal investigations involved in development of functional foods from edible Irish seaweeds

Task 1: Assessing of the bioactivity of edible Irish seaweeds	
Questions	Investigation
I. What is the antioxidant content of the seaweeds?	Extraction and analysis using total phenolic, flavonoid, condensed tannin and DPPH procedures
II. What is the antimicrobial activity of the seaweeds?	Extraction and analysis using microtitre antimicrobial analysis
III. What is the best solvent for extraction of phytochemicals?	Extract using different solvents such as methanol, ethanol and acetone, in order to determine efficacy of extraction
Task 2: Determining the effect of processing conditions on <i>Himanthalia elongata</i>	
Questions	Investigation
I. How does hydrothermal processing such as drying, boiling, steaming and microwaving affect the antioxidant constituents of the whole seaweed product?	Processing at different time/temperature combinations and determination of the antioxidant content of the whole seaweed product
II. How does hydrothermal processing such as drying, boiling, steaming and microwaving affect the antioxidant and antimicrobial constituents of the extracts?	Processing at different time/temperature combinations and determination of the antioxidant and antimicrobial content of the seaweed extracts
Task 3: Optimising processing conditions	
Questions	Investigation
I. How does drying at different temperatures affect the moisture and antioxidant capacity of the seaweed?	Drying at different temperatures and times and determination of moisture and antioxidant content of the seaweed
II. How does rehydrating at different temperatures affect the moisture and antioxidant capacity of the seaweed?	Rehydrating dried seaweed at different temperatures and times and determination of moisture and antioxidant content of the seaweed
III. Is it possible to model the dehydration and rehydration	Fitting a number of mathematical models to moisture data

processes?	
IV. What are the optimal drying pre-treatment and rehydration conditions?	Using Response Surface Methodology to study the effect of rehydration on the semi-dried optimised seaweed
Task 4: Developing functional foods from optimised seaweeds	
Questions	Investigation
I. What products can dried and rehydrated seaweeds be added to in order to enhance the phytochemicals and dietary fibre?	Incorporation of dried and rehydrated seaweeds into commonly consumed foods and determination of quality factors such as phytochemical and fibre levels, texture and colour
II. How does addition of the seaweed affect the sensory attributes?	Carry out sensory analysis studies to determine acceptability

CHAPTER 2

LITERATURE REVIEW

An overview of the existing published body of knowledge related to this thesis

2.1 Algae

Algae are classified as unicellular microalgae and macroalgae which are macroscopic plants of marine benthoses. Microalgae are found in both benthic and littoral habitats and also throughout the ocean waters as phytoplankton and macroalgae or seaweeds occupy the littoral zone. Seaweeds grow in the intertidal as well as in the sub-tidal area up to a certain depth where very little photosynthetic light is available (Dhargalkar and Pereira, 2005).

Seaweeds can be classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition (Darcy-Vrillon, 1993). The colour in case of green seaweeds is due to the presence of chlorophyll *a* and *b* in the same proportions as the ‘higher’ plants; beta-carotene (a yellow pigment) and various characteristic xanthophylls (yellowish or brownish pigments). The dominance of the xanthophyll pigment, fucoxanthin, is responsible for the colour of brown seaweeds. This compound masks the other pigments such as Chlorophyll *a* and *c* and other xanthophylls. Phycoerythrin and phycocyanin mask the pigments such as Chlorophyll *a* and beta-carotene and are responsible for the colour of red seaweeds (Dhargalkar and Pereira, 2005).

Seaweeds are a large and diverse group of organisms with many different life-cycle strategies, wide range of forms and occupy a range of habitats. Brown seaweeds, for example, range from a few centimeters in length to extremes of > 60 metres (m) in the Bull-Kelp forests of California, South America and Australia. The largest seaweeds in Ireland reach 4 – 5 at most (Morrissey *et al.*, 2001).

2.2 History of seaweed use

Most people unknowingly utilise seaweed products and extracts daily in the form of processed food items such as processed dairy, meat and fruit products and domestic commodities like paint, toothpaste, solid air fresheners and cosmetics. The importance of seaweeds for human consumption has been well known since 300 BC in China and Japan. These two countries are the major seaweed cultivators, producers and consumers in the world (Dhargalkar and Pereira, 2005). Marine algae have also been utilised in Japan as raw materials in the manufacture of many seaweed food products such as jam, cheese, wine, tea, soup and noodles (Nisizawa *et al.*, 1987). Estimates of seaweed intake in Japanese populations range from 3 – 13 grams (g) per day (Skibola, 2004). Low to zero seaweed intakes can be expected in Western countries, particularly North American populations (Yuan *et al.*, 2005).

Approximately 221 species of seaweeds are utilised commercially worldwide of which 65% are used as human food (Zemke-White and Ohno, 1999). Over the past few decades, the consumption of seaweed products has increased in European countries with approximately 15 – 20 types of edible algae are being commonly marketed (Dawczynski *et al.*, 2007). However, almost all of these seaweeds are in dried form. The total world seaweed production is estimated to be 8.5 million metric tonnes (excluding Antarctic seaweeds). Of this, 88.65% i.e. 7.5 million metric tonnes are produced by cultivation in an area of 200×10^3 hectares (ha), while the remaining 0.96 million metric tonnes are exploited from the natural seaweed beds worldwide Food and Agriculture Organisation (FAO, 2003). The estimated value of wide variety products that derived from the seaweeds is US \$5 – 6 billion worldwide. Food products for human consumption contribute approximately US \$5

billion of this. Substances that are extracted from seaweeds (hydrocolloids) account for a large part of the remaining billion dollars and smaller, miscellaneous uses, such as fertilizers and animal feed additives (FAO, 2004).

In Western countries the principal applications of seaweeds are as sources of hydrocolloids, thickening and gelling agents such as agar, alginates and carrageenans for various food and pharmaceutical uses. Seaweed genera used for extraction of alginates are *Ascophyllum*, *Durvillaea*, *Eclonia*, *Lessonia*, *Laminaria*, *Macrocystis*, *Sargassum* and *Turbinaria*. The major alginate producing countries are Scotland, Norway, China, Argentina, Australia, Canada, Chile, Mexico, Ireland, Japan and USA. Alginate production is valued US \$213 million annually (Dhargalkar and Verlecar, 2009). Table 2.1 outlines the various hydrocolloids from seaweed sources.

Table 2.1 Hydrocolloids from seaweed sources

Hydrocolloid	Source
Agar, agarose	Rhodophyta (<i>Gracilaria</i> , <i>Gelidium</i> , <i>Pterocladia</i> .)
Carrageenans	Rhodophyta (<i>Eucheuma</i> , <i>Chondrus</i> , <i>Hypnea</i> , <i>Gigartina</i>)
Fucoidan	<i>Laminaria religiosa</i> and other brown algae
Laminarin	Phaeophyta (<i>Laminaria japonica</i>)
Furcellaran	<i>Furcellaria lumbricalis</i>
Alginic acid (alginate)	Phaeophyta (<i>Macrocystis</i> , <i>Laminaria</i> , <i>Ascophyllum</i>)

Source: Venugopal (2009)

Agar is extracted from seaweeds by heating with water for several hours. The agar dissolves in the water and the mixture is filtered to remove the residual seaweed. The hot filtrate is cooled and forms a gel (jelly), which is broken into pieces, and

sometimes washed to remove soluble salts, and if necessary, it can be treated with bleach to reduce the colour intensity. The water is then removed from the gel and the product is milled to a suitable and uniform particle size (McHugh, 2003).

Alginates are naturally present in the cell wall of brown seaweeds (Kloareg and Quatrano, 1988). These polysaccharides show interesting rheological properties as they are able to enhance the viscosity of aqueous solutions at low concentrations and form gels or thin films (Vauchel *et al.*, 2009). The rationale behind the extraction of alginate from seaweed is to convert all the alginate salts in the seaweed to the sodium salt, dissolve this in water, and remove the seaweed residue by filtration. This can be carried out by various methods including addition of acids or calcium salt (McHugh, 2003).

Carrageenan is extracted from seaweed in a similar way to alginates. The seaweed is placed in an aqueous solution, the residue is removed by filtration and then the carrageenan is recovered from the solution (McHugh, 2003). The original source of carrageenan was from the Irish moss, *Chondrus crispus*. This seaweed is still used as a limited source but most carrageenans are now extracted from *Kappaphycus alvarezii* and *Eucheuma denticulatum*.

2.3 Overview of seaweed nutrition

Worldwide, around 32 Chlorophyta, 64 Phaeophyta and 125 Rhodophyta are being utilised. Of these, about 145 species (66%) are used for food purposes (Zemke-White and Ohno, 1999). From a nutritional point of view; edible seaweeds are low calorie foods with a high concentration of minerals, vitamins and proteins and a low content

of lipids which are in the range of 2.3 – 4.6% based on semidry sample weight (Dawczynski *et al.*, 2007). Seaweeds are excellent source of vitamins A, B1, B12, C, D and E, riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as calcium, potassium, sodium and phosphorus (Dhargalkar and Pereira, 2005). The quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetables mainly due to their high content in essential amino acids and relatively high level of unsaturated fatty acids. They have more than 54 trace elements, required for human body's physiological functions in quantities greatly exceeding vegetables and other land plants (Chapman and Chapman, 1980). However, compared to land plants, the chemical composition of seaweeds has been poorly investigated (Wong and Cheung, 2000).

In general, red and brown species of seaweeds demonstrate large differences in their protein content. Dawczynski *et al.* (2007) reported that the protein contents of seaweed products varied widely from $26.6 \pm 6.3\%$ in red algae, to $12.9 \pm 6.2\%$ in brown algae varieties. The same author also detected all essential amino acids in the seaweed species tested. Red seaweed species feature uniquely high concentrations of taurine when compared to brown algae varieties. Seaweed proteins, from red algae in particular, contain all essential amino acids, the levels of which are sufficient to meet dietary requirements (Dawczynski *et al.*, 2007). Therefore, red algae varieties represent an important source of protein. Wong *et al.* (2001) studied the nutritional values of seaweed protein concentrates isolated from two red and one green seaweed species and reported that the red species showed significantly higher protein extractability, protein digestibility and essential amino acids than the green species. Dawczynski *et al.* (2007) also reported that the fatty acid distribution of seaweed

products showed high levels of omega-3 fatty acids and demonstrated a nutritionally ideal omega-3/omega-6 free fatty acid ratio. Wong *et al.* (2001) also found that seaweed species *Undaria pinnatifida* and *Hijikia fusiforme* to be rich sources of omega-3 fatty acids. Seaweeds are valued for their high contents of polysaccharides (including agar, alginates and carageenans). The major components of seaweed polysaccharides are galactose, mannose and glucose. Thus seaweeds contain a significant amount of soluble polysaccharides which have potential function as dietary fibre (Venugopal, 2009).

The role of diet in human health is progressively gaining more attention over the last few years. Food is not only beneficial due to the presence of essential nutrients, but also due to the occurrence of other bioactive compounds which have been found to be important for health promotion and disease prevention. These beneficial effects can be attributed to the complex mixture of phytochemicals which possess antioxidant, antimicrobial, anticancer and antiviral activity. The compounds responsible for these activities include phenolic compounds, sulphated polysaccharides and organic acids and seaweeds are a rich source of such phytochemicals (Liu, 2003; Podsędek, 2007; Apostolidis *et al.*, 2008; Gupta and Abu-Ghannam, 2011).

2.4 Irish seaweeds

Ireland, with an extensive coastline of over 3000 km, has a long history of seaweed use that continues to the present day. Most of the Irish seaweed industry is concentrated on the West of Ireland which provides valuable employment in coastal areas that are geographically remote or otherwise severely disadvantaged

economically. Over 500 different species of seaweeds have been identified from Irish waters. Given the small area of the island, this is a relatively high number which represents the diversity of seaweeds in Ireland. One reason for this diversity is latitude, Ireland is located between 51 and 55° N, occupying a range of latitudes that straddle both the northern limit for some warm-water species, and the southern limit for some cold-water species (Morrissey *et al.*, 2001). Edible seaweeds are a renewable natural resource existing in large quantities however, there has been little exploitation and exploration of seaweeds (Ortiz *et al.*, 2006).

Ireland's seaweed and biotechnology sector is currently worth €18 million per annum. The product source is currently limited to the wild resource and product range is limited in the main to high volume, low value products such as animal feeds, plant supplements, specialist fertilisers and agricultural products. A smaller proportion goes into higher value products such as foods, cosmetics and therapies (Walsh and Watson, 2011). According to the Sea Change Strategy (2006), the Irish seaweed production and processing sector will be worth €30 million per annum by 2020.

Some of the common edible species of seaweed harvested from the West Coast of Ireland include *Laminaria* species (spp.), *Himanthalia elongata* and *Palmaria palmata*. Table 2.2 describes common edible seaweeds found around the Irish coastline and their traditional use as food sources.

Table 2.2 Some of the more common seaweeds found around the Irish coastline and worldwide traditional use as a food source

Common names	Taxonomic details	Uses
Kelps	<i>Laminaria</i> spp.	Commonly used in Asian cuisine for flavouring in sauces, stews and stocks
Kombu, kelp, tangleweed.	<i>Laminaria digitata</i>	Traditional boiled vegetable of the Hebrides. Usually sold dried in bundles, used as above.
Sweet kombu, sweet kelp, oarweed, sugar wrack, tangleweed.	<i>Laminaria saccharina</i>	Chewed as a sweet snack.
Wakame	<i>Undaria pinnatifida</i>	Used raw in salads and as a wrapping for savory parcels.
Laver, laverbread, sloke (Europe) Nori (Japan).	<i>Porphyra</i> spp.	Main ingredient of Welsh laverbread. Wrapping for sushi and savoury parcels in Japan.
Carrageen, Irish Moss, Dorset Moss, Iberian Moss.	<i>Chondrus crispus</i>	Gelling agent, traditionally boiled in milk for moulds and puddings. Boiled in milk with honey to sooth coughs & colds.
Dulse, dillisk, creathnach.	<i>Palmaria palmata</i>	Sold sundried and eaten in Ireland as a snack. Used raw in salads and slaws.
Pepper dulse	<i>Laurencia pinnatifida</i>	Used as a condiment.

Source: Hotchkiss (2007)

2.4.1 Brown algae

In Ireland 147 species of brown seaweeds have been reported, most of which are kelps and wracks. Brown seaweeds range from either olive-green to dark brown in

colour. These seaweeds appear to be brown due to a masking pigment known as fucoxanthin, which masks the green-colour of chlorophyll. Like terrestrial plants, chlorophyll is present in all seaweeds but is readily visible only in green seaweeds (Morrissey *et al.*, 2001). Brown seaweeds are usually large, and range from the giant kelp which is often 20 m long, to thick, leather-like seaweeds from 2 – 4 m long, to smaller species of 30 – 60 centimetres (cm) long (McHugh, 2003).

2.4.1.1 *Laminaria digitata*

Laminaria digitata is a very common kelp that survives at low water levels around the Irish coastline and can be seen in Fig. 2.1. It is commonly known as Oarweed or Tangleweed in Western countries and is called Kombu in Asia. *L. digitata* is golden-brown in colour, up to 2.5 m long and 60 cm wide across the frond. The frond is large, flat, rubbery and smooth with finger-like sections. Despite the massive resources of *L. digitata* off the coast of Ireland, only a tiny percentage is harvested either by hand or rake to supply the sea vegetable market (Morrissey *et al.*, 2001). The nutritional analysis of *L. digitata* is given in Table 2.3.



Fig. 2.1 Image of *Laminaria digitata*

Source: <http://www.algaebase.org>

2.4.1.2 *Laminaria saccharina*

Laminaria saccharina is another common kelp found in a range of habitats around the Irish coast. It is commonly known as Sweet Kombu or Sugar Kelp among other names. *L. saccharina* is easily recognizable by its relatively short yellow-brown stipe (< 60 cm), elongated frilly-edged and crinkled fronds that extend from 2 – 4 m in length and can be seen in Fig. 2.2. Small quantities of *L. saccharina* are hand-picked with a sharp knife at low tide and sold as a sea vegetable. Dried *L. saccharina* is arguably the most palatable of the kelps due to the presence of mannitol which imparts a sweet taste (Morrissey *et al.*, 2001). The nutritional analysis of *L. saccharina* is given in Table 2.3.



Fig. 2.2 Image of *Laminaria saccharina*

Source: <http://www.asturnatura.com>

2.4.1.3 *Himanthalia elongata*

Himanthalia elongata is often commonly referred to as Sea Spaghetti and is a common seaweed of a semi-exposed lower shore. This brown species of seaweed consists of long yellow-brown strap-like fronds which commonly grow up to 1 – 2 m

long and can be seen in Fig. 2.3 (Morrissey *et al.*, 2001). Relatively small amounts of *H. elongata* are hand-picked and sold as Sea Spaghetti and a nutritional breakdown of *H. elongata* is outlined in Table 2.3.



Fig. 2.3 Image of *Himanthalia elongata*

Source: <http://www.algaebase.org>

2.5 Red algae

Red algae show a wide range of colours ranging from a red or bright pink, to a dark purplish-brown, to almost black. The range of colours are a result of the presence of two extra pigments, phycoerythrin (red) and phycocyanin (blue) which mask the green chlorophyll and other photosynthetic pigments. 275 species of red algae are found in Ireland most of which are quite rare and particular to certain habitats or geographical regions (Morrissey *et al.*, 2001). Red seaweeds are usually smaller, generally ranging from a few centimeters to about a meter in length (McHugh, 2003).

2.5.1 *Palmaria palmata*

Palmaria palmata is a cold-water seaweed commonly known as Dulse and can be seen in Fig. 2.4 below. *P. palmata* has a reddish-brown to dull dark red colour which appears tough and almost leathery. The fronds of this plant are flat and 10 – 50 cm in length, the shape of the frond can vary considerably between an irregular fan-shape or a single frond with lateral marginal leaflets (Morrissey *et al.*, 2001).



Fig. 2.4 Image of *Palmaria palmata*

Source: <http://www.marlin.ac.uk>

P. palmata is commonly harvested in Ireland and other countries, sustainable harvesting technologies for this seaweed involve picking off the main mass of fronds, ensuring that the lower part of the plant and holdfast are left attached. This facilitates faster generation of the seaweed. Table 2.3 outlines the nutritional analysis of *P. palmata*.

2.5.2 *Chondrus crispus*

Chondrus crispus is a very common seaweed found on both sides of the Atlantic and can be seen in Fig. 2.5. It is commonly known as Carrageenan Moss or simply Carrageenan. *C. crispus* is a low bushy plant rarely exceeding 15 cm in height, with a variety of forms and colours, from dark red, brown-red, purplish green and brownish yellow to whitish-yellow when bleached by sunlight. *C. crispus* plants are short, with tough, flat fronds that divide regularly and often form a broad fan-shape tapering down to a narrow base and small disc shaped holdfast (Morrissey *et al.*, 2001).



Fig. 2.5 Image of *Chondrus crispus*

Source: <http://www.cybercolloids.net>

C. crispus is the most important seaweed in Ireland in terms of harvested tonnage and sustainable harvesting is in practice. Unknowingly, many people use or consume Carrageenans as well as other hidden seaweed extracts on a daily basis, in foods, toothpastes and various lotions and creams. Nutritional analysis of *C. crispus* is presented in Table 2.3.

2.6 Green algae

There have been 80 species of green algae found around the Irish coastline. Of the three algal divisions, green seaweeds show the least colour variation, usually being a bright green-grass colour. They owe this green colour to the presence of photosynthetic pigment chlorophyll, which is not masked by any other colour pigments as in red and brown algae (Morrissey *et al.*, 2001). Green seaweeds are similar in size to red species, ranging from a few centimeters to about a meter in length (McHugh, 2003).

2.6.1 *Enteromorpha spirulina*

The seaweeds from genus *Enteromorpha* are extremely common and known as Sea Grass. This seaweed is mid- to bright green with a maximum length of 15 – 30 cm with very narrow fronds as can be seen in Fig. 2.6 (Morrissey *et al.*, 2001).



Fig. 2.6 Image of *Enteromorpha spirulina*

Source: <http://www.mer-littoral.org>

Despite the abundance of *E. spirulina* around the Irish coastline there has been little use of it beyond some small scale drying as a sea vegetable and as a fertilizer. The nutritional analysis of *Enteromorpha* spp. is given in Table 2.3.

Table 2.3 Nutritional analysis of seaweed species

Nutritional Component	<i>L. digitata</i>	<i>L. saccharina</i>	<i>H. elongata</i>	<i>P. palmata</i>	<i>C. crispus</i>	<i>E. spirulina</i>
Protein (%)	8 – 14	6 – 11	6 – 13	12 – 21	11 – 18	10 – 18
Fat (%)	1	0.5	0.5	0.7 – 3	1 – 3	0.5 – 1.7
Carbohydrate (%)	48	61	65	46 – 50	55 – 66	48
Vitamin C (ppm)	12 – 18	13 – 18	11 – 18	150 – 280	10 – 30	40 – 122
Calcium (ppm*)	12400 – 13200	8910 – 9282	9110 – 9258	2000 – 8000	0.9 – 1.3%*	8200 – 9400
Iodine (ppm)	800 – 5000	800 – 4500	185	150 – 550	200 – 300	70
Iron (ppm)	50 – 70	22 – 40	31 – 40	56 – 350	170 – 210	152
Magnesium (ppm*)	6400 – 7860	5670 – 6944	5790 – 6448	0.2 – 05	6710 – 8351	2.6 – 2.8%*
Manganese (ppm)	1 – 16	1 – 16	1 – 18	10 – 155	2 – 28	1 – 12
Sodium (%)	2 – 5.2	3 – 3.4	3 – 3.8	0.8 – 3	2 – 2.6	7.3 – 8.4

*unit of measurement, except where indicated differently

ppm: parts per million

Source: Morrissey *et al.* (2001)

2.7 Bioactive compounds from seaweeds

Seaweeds are a known source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Bansemir *et al.*, 2006). Compounds with antiviral, antifungal, antimicrobial and antioxidant activities have been detected in green, brown and red algae (Vairappan *et al.*, 2001; Duan *et al.*, 2006; Cox *et al.*, 2010). The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that their cells have some protective antioxidative mechanisms and compounds (Matasukawa *et al.*, 1997). In recent years, many researchers have focused on marine algae and their constituents as nutraceuticals and functional foods for their potential health promotion mostly attributed to their omega-3 fatty acids, antioxidants, and other bioactives (Shahidi, 2008).

2.7.1 Phenolic compounds

The term ‘phenolic compound’ describes several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by, at least, one hydroxyl group. Distinctions are thus made between phenolic acids (benzoic or hydroxycinnamic acid derivatives), flavonoids, stilbenes, and lignans. The flavonoids may themselves be divided into flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids (Manach *et al.*, 2004).

In foods, phenolic compounds originate from one of the main classes of secondary metabolites in plants derived from phenylalanine and, to a lesser extent from tyrosine in some plants (Van Sumere, 1989; Shahidi, 2000; Shanidi, 2002). Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties (Duan *et al.*, 2006; Kuda *et al.*, 2007; Wang *et al.*, 2009; Cox *et al.*, 2010). These compounds may be classified into different groups as a function of the number of phenol rings that they contain, and of the structural elements that bind these rings to one another. Polyphenols represent a diverse class of compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavononols, chalcones and flavan-3-ols), lignins, tocopherols, tannins and phenolic acids (Shukla *et al.*, 1997). Together with long-chain carboxylic acids, phenolics are also components of suberin and cutin. These rather varied substances are essential for the growth and reproduction of plants and also act as antifeedant and antipathogens (Butler, 1992).

In addition, phenolic compounds also function as antibiotics, natural pesticides, signal substances for establishment of symbiosis with rhizobia, attractants for pollinators, protective agents against ultraviolet (UV) light, insulating materials to make cell walls impermeable to gas and water and as structural materials to give plants stability. The contribution of phenolics to the pigmentation of plant foods is also well recognized. Many properties of plant products are associated with the presence, type and content of phenolic compounds (Shihidi and Naczki, 2004). Dietary intake of phenolic compounds is greatly affected by the eating habits and preferences of individuals. The average daily intake of dietary polyphenols is about 1

g per person with the main sources being beverages, fruits, vegetables and legumes (Scalbert and Williamson, 2000).

2.7.2 Flavonoids

Flavonoids, the largest group of phenolic compounds, are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties (Kahkonen *et al.*, 1999). Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids (Ndhlala *et al.*, 2007). These compounds are formed via condensation of a phenylpropane (C6-C3) compound via participation of three molecules of malonyl coenzyme A, which leads to the formation of chalcones that subsequently cyclize under acidic conditions. Among the flavonoids, anthocyanins and catechins, known collectively as flavans are important, as these compounds are widely distributed across the plant kingdom and have a broad spectrum of chemical and biological activities, such as antioxidant activity and prevention against disease (Kahkonen *et al.*, 1999; Ross and Kasum, 2002; Shihidi and Naczki, 2004).

2.7.3 Tannins

Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants (Haslam, 1989; Waterman and Mole, 1994). Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolyzable compounds. Condensed tannins are oligomers and polymers of flavonoids, specifically flavan-3-ols (Shihidi and Naczki, 2004). Hydrolyzable tannins are gallic and/or eggallic acid which easily hydrolyze in acidic media, and

condensed tannins are polymeric flavonoids (Huang *et al.*, 2008). Many of these gallic acids are linked to sugar molecules (Shihidi and Naczki, 2004). In contrast to terrestrial tannins, phlorotannins are tannin compounds which have been found only in marine algae. Phlorotannins are formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga (Ragan and Glombitza, 1986; Waterman and Mole, 1994; Arnold and Targett, 1998).

Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (Ahn *et al.*, 2007). Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings (Hemat, 2007).

2.8 Antioxidant compounds

2.8.1 Oxidation

Lipids are present in almost all food raw materials, most of which are in the form of triglycerides and phospholipids. These fats are important substrates for oxidative deterioration which leads to the development of rancidity, food quality deterioration, off-flavours and other reactions. The process of autoxidation of polyunsaturated lipids in food involves a free radical chain reaction that is generally initiated by exposure of lipids to light, heat, ionizing radiation, metal ions or metalloprotein catalysts. Enzyme lipoxygenase can also initiate oxidation. The autoxidation process

occurs via three steps: initiation, propagation and termination (Shihidi and Nacz, 2004). Fig. 2.7 represents a general scheme for autoxidation of polyunsaturated lipids and their consequences in quality deterioration of food.

Reactive oxygen species (ROS) and their likely involvement in some human pathologies have attracted growing interest from the health sector over the last few decades. Oxidative stress, caused by and imbalance between antioxidant systems and the production of oxidants including ROS, seems to be associated with many multifactorial diseases, especially cancers (Kawanishi *et al.*, 2002), cardiovascular diseases (Sachidanandame *et al.*, 2005) and inflammatory disorders (Bodamyali *et al.*, 2000).

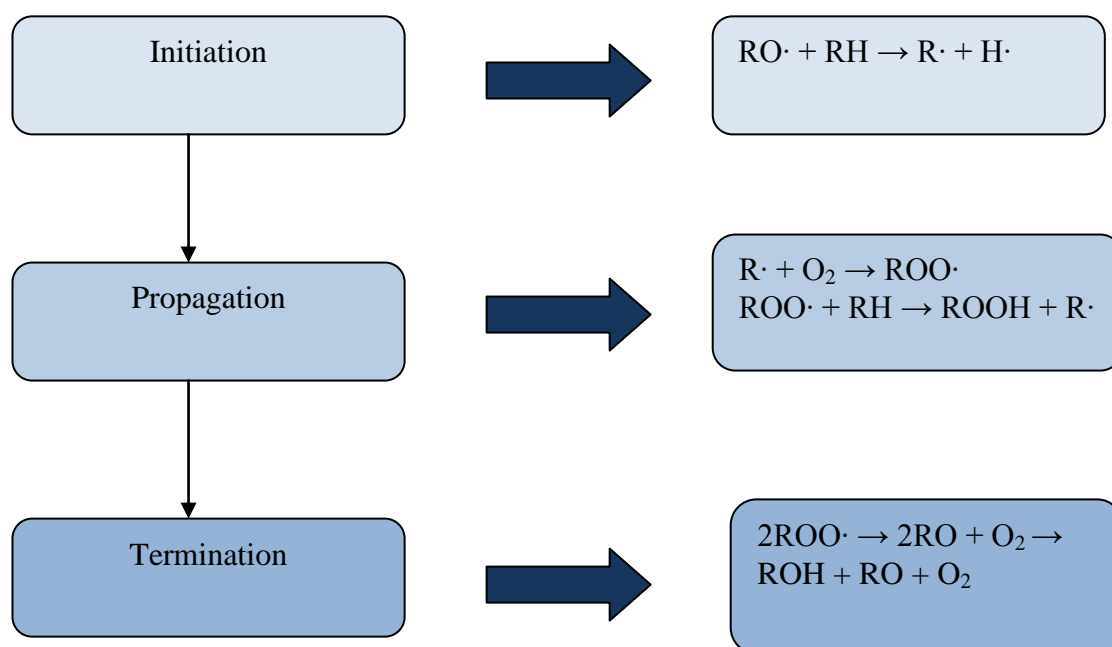


Fig. 2.7 General scheme for autoxidation of polyunsaturated lipids and their consequences in quality deterioration of food

Source: Shahidi and Nacz (2004) with some modifications

Diseases and damage caused by ROS can be seen in Fig. 2.8. The mechanisms by which these pathologies develop generally involve oxidative alteration of physiologically critical molecules, including proteins, lipids, carbohydrates and nucleic acids, along with modulation of gene expression and the inflammatory response (Laguerre *et al.*, 2007). ROS such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}), peroxy radical (ROO^{\cdot}) and nitric oxide radical (NO^{\cdot}), attack molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis and carcinogenesis (Valentão *et al.*, 2002; Fisch *et al.*, 2003; Nakamura *et al.*, 2003; Shon *et al.*, 2003).

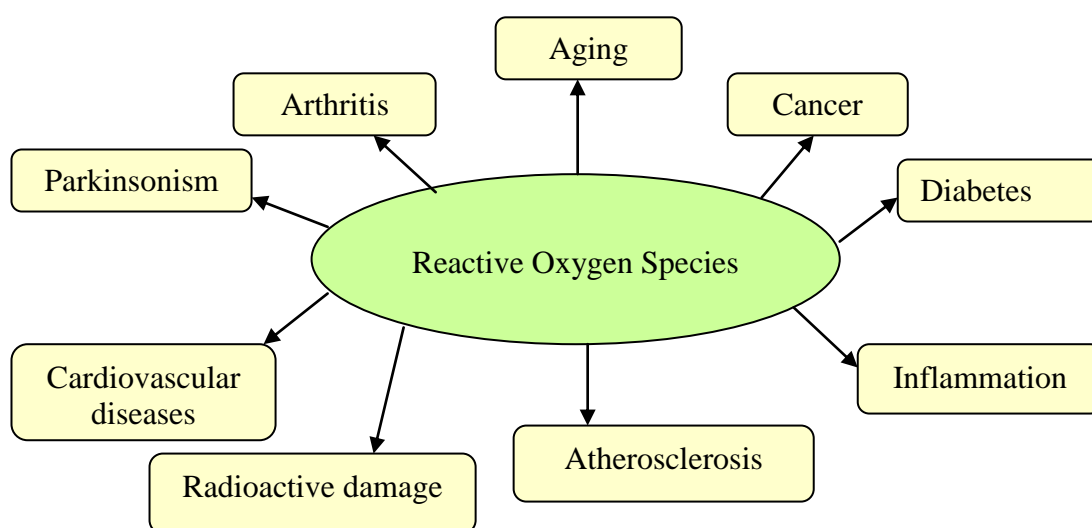


Fig. 2.8 Diseases and damages caused by reactive oxygen species

Source: Adapted from Shahidi and Naczki (2004)

2.8.2 Antioxidant compounds

Antioxidants are effective in protecting the body against ROS. According to Halliwell and Gutteridge (1990), the term ‘antioxidant’ refers to ‘a substance that

when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate'. Within the food industry, lipid peroxidation is a critical problem as it affects food quality and stability (Yu *et al.*, 2002). To prevent or delay peroxidation, antioxidants are utilised as has been the practice for over 50 years. Antioxidants act via different mechanisms by binding metal ions, scavenging free radicals and decomposing peroxides and hydroperoxides, among others (Moure *et al.*, 2001). Examples of mechanisms of antioxidant activity can be seen in Table 2.4.

Table 2.4 Mechanisms of antioxidant activity

Antioxidant class	Mechanism of antioxidant activity	Examples of antioxidants
Proper antioxidants	Inactivating lipid free radicals.	Phenolic compounds, flavonoids, tocopherols
Hydroperoxide stabilizers	Preventing decomposition of hydroperoxides into free radicals.	Phenolic compounds
Synergists	Promoting activity of proper antioxidants.	Citric acid, ascorbic acid
Singlet oxygen quenchers	Transforming singlet oxygen into triplet oxygen.	Carotenoids
Substances reducing hydroperoxides	Reducing hydroperoxides in a non-radical way.	Proteins, amino acids
Metal chelators	Binding heavy metals into inactive compounds.	Flavonoids, phosphoric acid, maillard components, citric acid

Source: Adapted from Pokorny (2001) and Shahidi and Naczk (2004)

Interest in new sources of natural antioxidant molecules has increased in recent years in order to reduce the use of synthetic forms such as Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) where there may be safety and toxicity problems (Duan *et al.*, 2006). According to Laguerre *et al.* (2007), it has been confirmed that exogenic antioxidants, especially supplied from foods, are essential for counteracting oxidative stress. These antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols, ascorbic acid and carotenoids.

Aromatic herbs, tea, grapes and derived products, citrus peel and seeds are amongst the most studied natural antioxidants. Despite major research, extensive knowledge has not yet been gained into the power of antioxidants derived from plants, nor has their potential been substantially tapped (Laguerre *et al.*, 2007). Among natural antioxidants, phenolic antioxidants are in the forefront as they are widely distributed in the plant kingdom. All of the phenolic classes, for example, phenolic acids, anthocyanins and flavonoids, have the structural requirements of free radical scavengers and have potential as food antioxidants (Bandoniene and Murkovic, 2002). Furthermore, antioxidants from natural sources can increase the shelf-life of foods (Schwarz *et al.*, 2001) and also act as a defense mechanism of living cells against oxidative damage (Vimala *et al.*, 1999).

Natural antioxidants are not limited to terrestrial sources. Plants in general and algae in particular are good sources of natural antioxidants. During the photosynthesis process seaweeds absorb solar light which is converted into chemical energy, later used in the conversion of carbon dioxide into carbohydrates, and at the same time,

generating molecular oxygen, which can reach locally high concentration levels. As oxygen is easily activated by UV radiation or heat from sunlight into toxic ROS, plants and algae have developed a protective mechanism which consists of antioxidant compounds which are able to minimize the concentration of these ROS (Lu and Foo, 1995).

Recently, reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007; Cox *et al.*, 2010). Chlorophylls, carotenoids, tocopherol derivatives such as vitamin E, and related isoprenoids, that are structurally related to plant-derived antioxidants, were found in some marine organisms (Takamatsu *et al.*, 2003). Antioxidant activity of marine algae may arise from pigments such as chlorophylls and carotenoids, vitamins and vitamin precursors including α -tocopherol, β -carotene, niacin, thiamine and ascorbic acid, phenolics such as polyphenolics and hydroquinones and flavonoids, phospholipids particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, which directly or indirectly contribute to the inhibition or suppression of oxidation processes (Shahidi, 2008).

2.9 Antimicrobial compounds

Food poisoning is a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety regulators and regulatory agencies are continuously concerned with the high and growing number of illness or outbreaks caused by some pathogenic and spoilage microorganisms in foods. Recently, consumers are demanding foods which are fresh, natural and minimally processed. Along with this, consumers are also concerned about the safety

of foods containing synthetic preservatives. This has put pressure on the food industry and has fuelled research into the discovery of alternative natural antimicrobials (Shan *et al.*, 2007).

Refrigerated, ready-to-eat products have become increasingly popular in recent years due to their convenience. Many food spoilage and pathogenic bacteria spoil such foods, reducing their shelf life and can also lead to food poisoning of the consumer. In addition to microbial contamination, all packed and refrigerated foods also undergo gradual changes during storage, due to autooxidation which releases ROS, including free radicals such as superoxide anion and hydroxyl radicals, and non-free radical species like singlet oxygen and hydrogen peroxide into the food (Haliwell and Gutteridge, 1989; Yildirim *et al.*, 2000; Devi *et al.*, 2008). These ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidation products such as heptanol and hexanal which contributes to oxidative rancidity, thus deteriorating food flavour (St Angelo *et al.*, 1987; Ladikos and Lougovois, 1990). As a means to overcome these issues, a range of synthetic antimicrobial agents have been used such as sodium benzoate, sodium nitrite and calcium benzoate and also synthetic antioxidants, BHA and BHT. However these preservatives can cause liver damage and are suspected to be mutagenic and toxic (Duan *et al.*, 2006; Devi *et al.*, 2008).

Plant products with antimicrobial properties have obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth (Lanciotti *et al.*, 2004). Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne

pathogens (Askun *et al.*, 2009; Jeong *et al.*, 2009; Nedorostova *et al.*, 2009). However, little information is available on the effect or potential of the preservative and antimicrobial role of seaweed extracts.

In the marine ecosystem, seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and yeasts (Vairappan *et al.*, 2001). Seaweeds are a rich source of structurally diverse secondary metabolites including terpenes, acetogenins, alkaloids and polyphenolics, with many of these compounds being halogenated (Watson and Cruz-Rivera, 2003). Halogenated secondary metabolites, such as bromophenols and phlorotannins with diverse structural features are known to be produced by algae (Xiaojun *et al.*, 1996; Li *et al.*, 2007; Oh *et al.*, 2008). The occurrence of bacterial biofilms on seaweed surfaces is ubiquitous, and has many negative effects on the seaweed, such as increased drag, competition for nutrients and blocking light and gaseous exchange. The functions of these secondary metabolites include defense against herbivores, fouling organisms and pathogens and they also play a role in reproduction, protection from Ultra Violet (UV) radiation and as allelopathic agents (Hay, 1996; Watson and Cruz-Rivera, 2003). Physical stress such as desiccation, UV and visible light and nutrient availability are able to alter the secondary metabolites in seaweeds (Watson and Cruz-Rivera, 2003). The oceans have enormous biodiversity and potential to provide novel compounds with commercial value (Hay, 1996; Smit, 2004).

Although antimicrobial studies from the extracts of several seaweeds have been studied, cases of the extracts being added to food products as a means to enhance the safety are not widely available (Gupta and Abu-Ghannam, 2011). Bioactive

compounds from seaweeds have shown antimicrobial action against a number of Gram-positive and negative bacteria (Han *et al.*, 2005; Kim *et al.*, 2008; Gupta *et al.*, 2010; Manivannan *et al.*, 2011). The compounds responsible for antimicrobial activity of seaweeds are terpenes, phenolic or lipophilic in nature. Phlorotannins extracted from brown seaweeds have been reported to have antibacterial activities against Gram-positive and negative bacteria (Nagayama *et al.*, 2002; Sandsdalen *et al.*, 2003). A compound isolated from brown seaweed, *Fucus vesiculosus*, exhibited bactericidal activity against both Gram-positive and the Gram-negative bacteria (Sandsdalen *et al.*, 2003) while compounds from red algae, *Rhodomela confervoides* has also exhibited bactericidal activity against Gram-positive and the Gram-negative bacteria (Han *et al.*, 2005). These findings suggest a potential use of seaweed extracts as natural preservatives in the food industry or as antibacterial drugs.

2.10 Effect of food processing on phytochemicals present in plant products

The presence and diversity of phytochemicals in vegetables are important factors for human health. The phytochemical contents in untreated vegetables have been the most studied. Since a large part of ingested vegetables are generally thermally processed prior to consumption, it is also important to investigate how the processing affects the levels of these compounds (Volden *et al.*, 2009). Many plant-based foods can be eaten raw or after being processed. Processing can be performed in various ways but, for vegetables, most common are steaming, boiling and microwaving which would be similar for seaweeds. These thermal processes would bring about a number of changes in physical characteristics and chemical composition of the vegetables (Zhang and Hamauzu, 2004). Reports on the effects of thermal processing on the antioxidant compounds in vegetables have been inconclusive. There are

reports demonstrating an enhancement or no change in antioxidant activity of vegetables (Gahler *et al.*, 2003; Turkman *et al.*, 2005) while others have indicated a deterioration of activity after thermal treatments (Ismail *et al.*, 2004; Zhang and Hamazu, 2004).

Processing of vegetables for consumption exposes the phytochemicals present to detrimental factors that may lead to alterations in concentrations and health related quality. For example; wet-thermal treatment causes denaturation of enzymes that can catalyse breakdown of nutrients and phytochemicals. On the other hand, processing by heat can result in reduction of nutritional constituents by leaching or due to thermal destruction (Rungapamestry *et al.*, 2007). There have been few reports on the effect of hydrothermal processing on seaweeds. Turkmen *et al.* (2005) revealed that different heating methods (boiling, steaming and microwaving) caused losses of phenolics from squash, peas and leek. However, under similar conditions, an increase in the phenolic content of vegetables such as green beans, peppers and broccoli was reported (Turkman *et al.*, 2005). Watchtel-Galor *et al.* (2008) found that steaming and microwaving led to losses in the total phenolic content of broccoli, choy-sum and cabbage, although steaming had significantly lower losses than microwaved samples. Volden *et al.* (2009) also reported loss of phytochemicals in steamed cauliflower (19%), whereas Cliszcyńska-Świgło *et al.* (2006) found a 52% increase in the total phenolic content of steamed broccoli, explaining this by enhanced extractability due to disruption of the polyphenols-protein complexes.

Epidemiological studies have shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases such as

cancer, cardiovascular disease, cataract and muscular degeneration (Heim *et al.*, 2002; Hunter and Fletcher, 2002; Cheung *et al.*, 2003; Zhang and Hamauzu, 2004). Treatment of vegetables for consumption exposes the phytochemicals to detrimental factors that may lead to alterations in concentrations and health related quality (Volden *et al.*, 2009). In reality, only a small amount of fruits and vegetables are consumed in their raw state, as most of them need to be processed for safety, quality and economic reasons. The evaluation of the influence of food processing is a key factor while establishing technological conditions that enable to preserve or improve original activity and bioavailability of naturally occurring antioxidants (Kusznierewicz *et al.*, 2008). In the development of new food products from seaweeds it will be important to ensure that the processing conditions utilised do not adversely affect the contents of bioactive compounds, in order to produce functional food products with nutraceutical properties.

2.11 Food dehydration and rehydration

Being marine in nature, seaweeds contain a large amount of water. When fresh, they contain 75 – 85% water and 15 – 25% organic components and minerals. Dry matter accounts for 65 – 85% organic substances and 30 – 35% is ash (FAO 2005). The traditional process to preserve seaweeds is by sun drying (Lim and Murtijaya, 2007) as several seaweeds are perishable in their fresh state and could deteriorate within a few days after harvest. Drying is one of the most common food processing methods that can be used to extend its shelf-life and to achieve the desired characteristics of a food product. Reducing the water activity (a_w) of food via this process can minimise deterioration from chemical reactions and microbial activity (Chiewchan *et al.*, 2010).

Drying helps to retard microbial growth, reduce the bulk handling thereby facilitating transportation and allows their usage during the off-season (Mota *et al.*, 2009). However, other processes, promoted by high temperatures, can occur simultaneously with moisture removal during drying, resulting in undesirable alterations of certain characteristics of the material, such as shrinkage and colour changes (enzymatic and non-enzymatic browning) (Maskan, 2001). During air-drying, spatial conformation of food material components can be partially altered by water flux. In addition, there is a partial destruction of tissue structure, which results in water permeability. As a result, rehydration ability can also decrease, and there can be changes in the texture (Krokida *et al.*, 2000; Lewicki and Jakubczyk, 2004).

The traditional way to preserve plant products is by sun drying. However, enzymatic and/or non-enzymatic processes that may occur during drying of the fresh plant tissues may lead to significant changes in the composition of phytochemicals (Capecka *et al.*, 2005). Generally these processes cause negative attributes to the final food product, but studies by Nicoli *et al.* (1999) showed that the overall antioxidant properties of certain foods may instead be enhanced due to improvement in the antioxidant properties of naturally occurring antioxidants and the formation of Maillard reaction products.

The food sector of the Irish seaweed industry consists mainly of micro-enterprises employing five people or less, with very limited automation in harvesting, drying or processing (Walsh and Watson, 2011). Seaweeds are among the plants which are traditionally sun dried. This is carried out by spreading the harvested wet seaweed over a net or a tarpaulin on the ground. The bulk of the seaweed industry utilises

drying as a method of preservation for a number of reasons including the fact that it does not require extensive training, or purchase of expensive equipment (Walsh and Watson, 2011). In Ireland, drying of seaweeds for the production of different grades of seaweed meal is carried out by drying in rotary dryers heated by coal slack fired kilns (www.cleanerproduction.ie).

Different drying methods have been found to greatly affect the nutritional composition of the brown seaweed, *Sargassum hemiphyllum* (Chan *et al.*, 1997). Wong and Cheung (2001) studied the effects of oven-drying and freeze-drying on protein extractability of three subtropical brown seaweeds. They found that oven-drying significantly improved the protein extractability and the protein quality. However, the long drying times at relatively high temperatures during the falling rate periods often lead to undesirable thermal degradation of the finished products (Mousa and Farid, 2002).

Dehydrated food products are usually rehydrated before consumption. Rehydration is a complex process intended to restore the properties of the fresh product by contacting dehydrated products with a liquid phase. The process is composed of three simultaneous steps: (1) absorption of water into the dry material, (2) swelling of the rehydrated product, and (3) loss or diffusion of soluble components (Marin *et al.*, 2006; Lee *et al.*, 2006). Typically, a higher rate of water absorption is observed during initial stages which then decline until equilibrium is achieved. Water temperature is the most important factor influencing the rehydration rate and generally more rapid rehydration is obtained at higher water temperatures. Treatments such as drying and rehydration produce changes in the structure and

composition of product tissues. Rehydration or blanching is carried out to make vegetables more palatable but studies have shown that phenolic compounds are sensitive to heat, whereby blanching and boiling of vegetables for few minutes could cause a significant loss of phenolic content which can leach into boiling water (Amin *et al.*, 2006). Since seaweeds are most commonly consumed after rehydration, it will be important to investigate the effect of rehydration on the phytochemicals which are present.

2.12 Dietary fibre

Dietary fibre is the major component of seaweeds; the term ‘dietary fibre’ was first used in 1953, in place of ‘crude fibre’, to refer to the non-digestible residue in foods (Potty, 1996). Dietary fibre in seaweeds is mainly composed of four families of polysaccharides; laminarans, alginates, fucans and cellulose. Laminarans are reserve polysaccharides found in brown algae and are composed of (1,3)- β -D-glucose with some (1,6)-linkages in which some of the reducing ends are replaced with mannitol. The major matrix component of brown seaweeds is a gelling polyuronide, alginate, consisting of alternating sequences of β -(1,4)-D-mannuronic acid, its C5 epimer α -(1,4)-L-guluronic acid and 20 – 30 units of uronic acids (Jiménez-Escrig and Sanchez-Muniz, 2000).

Fucans are polysaccharides which can be classified into three major groups; fucoidans, xylofucoglycuronans and glycorunogalactofucans (Jiménez-Escrig and Sanchez-Muniz, 2000). Cellulose constitutes cell walls of brown algae, and the cell walls of red algae are composed of sulphated galactans (carrageenans and agar), xylans, mannans and cellulose (Lahaye, 1991). Green seaweeds contain starch,

cellulose, xylans, mannans and ionic polysaccharides which contain sulphate groups and uronic acids. Lahaye (1991) reported that rhamnose, xylose, galactose and arabinose are also found in green algae.

Polysaccharides, not only function as dietary fibre, but they also contribute to the antioxidant activity of marine algae. A number of authors have reported antioxidant activity of algal polysaccharides (Xue *et al.*, 2001; Ruperez *et al.*, 2002; Zhang *et al.*, 2003). Consumption of seaweeds can increase the intake of dietary fibre and lower the occurrence of some chronic diseases such as diabetes, obesity, heart diseases and cancer which are associated with low fibre diets of the Western countries (Southgate, 1990). Dietary fibre can be divided into soluble and insoluble fractions. The viscosity of soluble fibre is responsible for slower digestion and absorption of nutrients, and lower levels of blood cholesterol and glucose. Insoluble dietary fibre is characterized by its ability to increase fecal bulk and decrease intestinal transit time (Baghurst *et al.*, 1996; Potty, 1996).

Seaweeds are rich in fibre (33 – 50 g/100 g db), particularly soluble fractions (50 - 85% of total dietary fibre content), therefore they can be exploited in order to enrich the fibre contents of foods which are generally low in this component (Jiménez-Escrig and Goni, 1999; Jiménez-Escrig and Sánchez-Muniz, 2000; Rupérez and Saura-Calixto, 2001). Table 2.5 contains a comparison of the fibre content of seaweeds and other whole foods. For example, fishery products, which otherwise possess high nutritional properties, are poor in fibre contents. Such products can be enriched with seaweed to improve the nutritional and functional properties such as water-binding and gelling. The modification of the emulsifying capacity by the

addition of fibre is important to the sausage and fish-processing industries (Borderías *et al.*, 2005; Venugopal, 2009).

Table 2.5 Comparison of fibre in seaweeds and other whole foods (% per 100 g fresh weight (fw))

Seaweeds	Fibre (%)	Whole Foods	Fibre (%)
<i>Himanthalia elongata</i>	9.8	Lentils	8.9
<i>Laminaria digitata</i>	6.2	Brown rice	3.8
<i>Palmaria palmata</i>	5.4	Bananas	3.1
<i>Porphyra</i> spp.	3.8	Cabbage	2.9
<i>Ulva</i> spp.	3.8	Prunes	2.4
<i>Undaria pinnatifida</i>	3.4	Porridge	0.8

Source: Institute de Phytonutrition database (2004) and McCance *et al.* (1993)

As consumers are becoming aware of the relationship between health and diet, the demand for functional foods is increasing. The high content of fibres and mineral elements in seaweeds, advocates their use as a means to improve the fiber content and reduce the salt content of many food products. The addition of seaweed, as a functional ingredient, can also help to overcome the technological problems associated with some products, including fat and water binding properties. The supplementation of seaweeds in foods could also increase consumption amongst the non-seaweed consuming population (Gupta and Abu-Ghannam, 2011). Dietary fibre from different algal sources is known for the capacity to lower serum cholesterol levels (Jiménez-Escrig and Sánchez-Muniz, 2000; Ginzberg *et al.*, 2000) and has the potential to be used as natural antioxidants by the food industry (Jiménez-Escrig *et al.*, 2001; and Rupérez *et al.*, 2002).

2.13 Nutraceuticals and Functional foods

Economic, cultural and scientific development has given rise to important changes in our food habits and life-style. Diets in developed countries are highly calorific, rich in saturated fats and sugars, while the consumption of complex carbohydrates and dietary fibre is low. This fact, together with a decrease in physical activity, has given rise to an increase of obesity along with a rise in the incidence of heart disease, diabetes and hypertension in the population (Geslain-Lanéelle, 2006).

Epidemiological studies on the relationship between dietary habits and disease risk have shown that food has a direct impact on health. It is generally accepted that plant derived foods such as wine, fruits, nuts, vegetables, grains and legumes exert some beneficial effects on human health, particularly age-related diseases. This fact has encouraged several health organizations such as the WHO and FAO around the world to recommend an increase in the intake of plant derived foods (Espín *et al.*, 2007). Eating patterns of people all over the world have undergone marked changes, due to globalisation of markets along with innovation in food technology (Subba-Rao *et al.*, 2007). Marketing studies carried out by diverse industries have shown consumers increasing demand for health-promoting food products, as well as for non-food products (i.e. diabetics and pharmaceuticals) containing the active principles present in these health-promoting foods. Many food bioactive constituents have been commercialised in the form of pharmaceutical products such as pills, capsules, solutions and gels, that incorporate food extracts or phytochemical-enriched extracts to which a beneficial physiological function has been directly or indirectly attributed (Espín *et al.*, 2007).

This range of new products cannot truly be classified as ‘food’ therefore a new hybrid term between nutrients and pharmaceuticals, ‘nutraceuticals’, has been coined to designate them (Espín *et al.*, 2007). According to Zeisel (1999) nutraceuticals are defined as ‘diet supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods’.

The food industry is currently directing new product development towards the area of functional foods and functional food ingredients due to consumers demand for healthier foods. Table 2.6 outlines some of the key consumer trends driving the demand for functional foods. The interest in developing these foods is thriving, driven largely by the market potential for foods that can improve the health and well-being of consumers (Charalampopoulos *et al.*, 2002). According to a widely accepted definition, a functional food is any modified food that may provide a health benefit beyond the nutrients it contains (FDA, 2004). These healthy foods include products with reduced fat, sugar or salt, fortified with vitamins, minerals, phytochemicals and containing probiotics (Manzi *et al.*, 2007). These products are often marketed as promoting health or reducing the risk of disease. There is therefore a potential niche in the market for development of functional foods from seaweeds in order to capitalise on their rich phytochemical and dietary fibre levels.

Table 2.6 Key consumer trends driving demand for functional foods

Consumer Trends	Implications
Ageing Population	Increased life expectancy
Demand on Health Services	Increased economic burden
Awareness of Diet/Health Relationship	Increased demand for healthier foods Increased interest in products that may reduce the symptoms of aging
Proactive about Health	Increased demand for products which will prevent disease

Source: Adapted from Gray et al. (2003)

According to Leatherhead Food Research Association (2004) and Hallem and Young (2000), gut benefit foods and beverages have dominated the global functional food market in terms of both increased volume of sales and new product development.

Fig. 2.9 illustrates consumer preference of functional food categories.

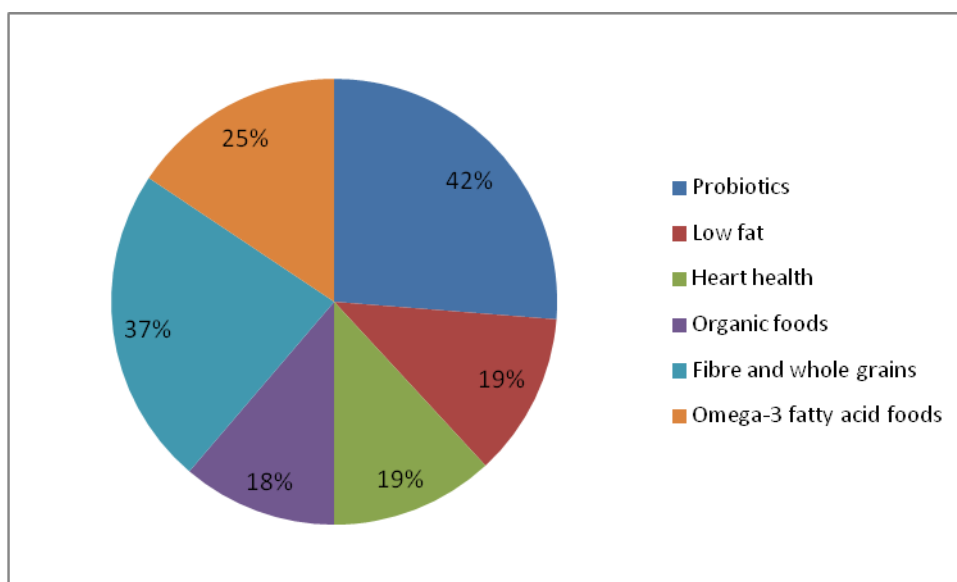


Fig. 2.9 Consumer preference of food categories related to health and wellness

Source: Adapted from Hansen (2006)

The current world market for functional foods and nutritional supplements is highly dynamic and is estimated to be US \$100 billion, with an annual growth potential of 20% (Kumara *et al.*, 2005; Aluko, 2006). Japan is leading the market for functional foods, with an estimated sale of US \$11.7 billion in 2003 with over 200 functional foods being marketed under the FOSHU legislation (Makhal *et al.*, 2004). The European functional food market was worth US \$3.49 billion in 2007; the largest segment being gut health-specific products (Kumara *et al.*, 2005; Aluko, 2006).

2.14 Development of new functional foods and nutraceuticals

Traditional economic demand analysis states that consumer food choices are primarily based on the price attribute; however, increasingly, consumers in developed countries are taking into account in food purchase decision other attributes such as quality, healthiness, convenience (Brunso *et al.*, 2002). The extent to which consumers find health claims appealing depends on a number of factors, including the content and format of the message (Mazis and Raymond, 1997). There are basically two types of health claims: ‘enhanced function’ and ‘reduced disease risk’. ‘Enhanced function’ claims relate to the consumption of a food or food component that contributes beneficially to health. ‘Reduced disease risk’ claims relate to the consumption of a food or food component that helps to reduce the risk of a specific disease or an undesirable health condition. The use of one type of claim depends on which has the greater persuasive impact on consumers (Van Kleef *et al.*, 2005).

According to Krishnamurthy *et al.* (2001) and Levin *et al.* (1998), in the context of attribute framing, people react more positively to positive than negative framing.

Therefore, enhanced function claims might be more appealing to consumers than reduced disease risk claims, because the former evoke positive associations from memory (Van Kleef *et al.*, 2005). Factors which influence consumer's selection of nutraceuticals can be seen in Table 2.7.

Table 2.7 Factors influencing consumer's selection of nutraceuticals

Factors Influencing Selection of Nutraceuticals
Awareness of nutritional significance
Cultural considerations
Frequency of consumption to obtain the desired effect
Health consciousness
Medical authorization
Mode of consumption
Nature of product and its ingredient
Sensory properties
Source (synthetic or natural)
<i>Source: Adapted from Venugopal (2009) and Schaafsma (2004)</i>

For a food product to gain acceptability, two important criteria need to be satisfied, namely, its sensory and nutritional properties. The functional value of any food, therefore, should be viewed from the point of view of both nutritional functionality and sensory functionality (Venugopal, 2009).

2.15 Potential new functional foods from seaweeds

Seaweed products may hold potential in the functional food market, particularly as sources of polysaccharides, dietary fibre, vitamins and micronutrients (Walsh and Watson, 2011). Marine products, due to their phenomenal biodiversity, are attractive not only as nutritious food items but also as a treasure house of novel, biologically

active compounds. The global markets for marine biotechnology products and processes were US \$2.4 billion in 2002, a 9.4% increase from 2001 (Venugopal, 2009). Seaweeds contain numerous bioactive compounds with a variety of potential health effects as seen in Table 2.8. There is great potential for these bioactive compounds to be extracted from seaweeds and added to foods or for the whole seaweed to be utilised in order to develop new functional foods and nutraceuticals.

Traditionally, and from a basic knowledge, phenolic compounds have been considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilisation of vitamins and minerals, reducing the nutritional values of foods. However, the recent recognition of their antioxidant properties reduced the investigations of their adverse health effects. The presence of phenolic compounds in the diet is beneficial to health due to their chemopreventive activities against carcinogenesis and mutagenesis. The health effects of phenolic compounds depend on the amount consumed and on their bioavailability (Chung *et al.*, 1998; Shan *et al.*, 2007).

Table 2.8 Some bioactive compounds from seaweeds and their functions

Seaweed species	Ingredient	Potential Health Effects
<i>Sargassum vulgare</i>	Alginic acid, xylofucans	Antiviral activity
<i>Himanthalia elongata</i>	PUFAs, α -tocopherol, sterols, fibre	Reduction of total and low density lipoprotein (LDL) cholesterol
<i>Undaria pinnatifida</i>	PUFAs, α -tocopherol, sterols. Fibre, folate, fucodanthin	Reduction of total and LDL cholesterol and certain types of cancer, antiviral activity
<i>Chondrus crispus</i>	PUFAs, α -tocopherol, sterols. fibre, folate, fucodanthin	Reduction of total and LDL cholesterol, reduction of cardiovascular disease
<i>Ulva</i> spp.	Sterol	Reduction of total and LDL cholesterol
Various seaweeds including: <i>Sargassum lomentaria</i> , <i>S. latiuscula</i> , <i>S. ringgoldianum</i> , <i>Rhodomela confervoides</i> , Wakame	K-carrageenan, ι -carrageenan, λ -carrageenan, fucoxanthin, fucoidan	Obesity control, apoptosis of cancer cells, induction of docosa hexanoic acid (DHA), antitumor activity
<i>Fucus vesiculosus</i>	Hyperoxaluria	Sulfated polysaccharides are potential blood anticoagulant agents

Source: Venugopal (2009)

Some companies have exploited seaweed extracts in functional foods, Diana-Naturals have marketed an anti-atherosclerosis capsule manufactured from *Fucus vesiculosus* and AHD International have also marketed Fucoxanthin from *Undaria pinnatifida* as a micronutrient supplement that can be added to functional beverages (Walsh and Watson, 2011).

Currently, most Irish seaweeds are washed, graded, dried and packaged with little done to increase consumer appeal or interest and dried seaweed may appear inaccessible and unfamiliar to a wide range of consumers (Watson and Walsh, 2011). The 2011 Bord Iscaigh Mhara (BIM) *Market Analysis towards Further Development of Seaweed Aquaculture in Ireland* report stated that it is necessary to innovate new products and formats such as “ready to eat” snacks and drinks, vitamin and mineral fortified products, culinary and foodservice ingredients such as stocks, soup bases, vegetarian foods and appealing novel alternatives to popular Asian products like Nori and Hijiki. The report also states that there must be a science-based approach to the development and marketing of novel seaweed products as currently there is little effort made to capitalise on the nutritional content of seaweed.

There have been reports where seaweeds were added to food products in order to exert functional or structural properties. Fernández-Martín *et al.* (2009) incorporated *H. elongata* into pork sausages in order to replace animal fat and studied the effects on meat batter gelation and López-López *et al.* (2011) added *U. pinnatifida* to beef patties in order to reduce salt and fat levels. Prabhasankar *et al.* (2009a and 2009b) added *U. pinnatifida* and *Sargassum marginatum* to pasta to increase antioxidant levels. Green seaweed, *Monostroma nitidum* was added to noodles in order to

develop a new product and found that cooking yields were improved up to one third (Chang and Wu, 2008). Choi *et al.* (2012) incorporated brown seaweed *Laminaria japonica* into pork patties in order to reduce fat content and increase dietary fibre levels and the resulting product had better sensory scores for overall acceptability when compared to the control. Cofrades *et al.* (2011) reported that *H. elongata* added to restructured poultry was also found acceptable by a sensory panel. The use of seaweeds as food ingredients is thus of indubitable interest from the standpoints of nutrition and technology (Cofrades *et al.*, 2008).

2.16 Development of potential new functional foods from Irish seaweeds

Seaweeds are a plentiful renewable natural resource in Ireland. *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina* are common species of seaweeds found in abundance around the Irish coastline yet the wealth of this natural resource has been relatively untapped. Many researchers have reported on the antioxidant and antimicrobial activity of seaweeds (Gonzalez del Val *et al.*, 2001; Kuda *et al.*, 2007; Ganesan *et al.*, 2008; Wang *et al.*, 2009). However, reports on the antioxidant and antimicrobial activity of seaweed extracts from Ireland are very limited. The Irish Marine Institute initiated ‘*A Marine Knowledge, Research and Innovation Strategy for Ireland 2007 - 2013*’ in 2007. The strategy included seaweed research priorities such as extraction, purification and identification of bioactive compounds from seaweed, along with development of functional foods from whole seaweed. In 2011, the Irish Government initiated the first steps in developing an ‘*Integrated Marine Plan for Ireland*’ in order to introduce new ways, new approaches and new thinking to promote a public debate on how best to harness our ocean wealth. The plan stated that seaweeds are increasingly being recognised as a

source of novel bioactive compounds with applications in pharmaceutical and functional foods, many of which are only being discovered. It was also noted that in order to capitalise on the potential for increased profitability in Ireland, the existing Irish seaweed processing sector must move up the value chain into the identified opportunity areas such as nutraceuticals. To achieve this, the sector, in association with the agencies and research providers needs to identify specific market opportunities, innovate and introduce greater automation, including new processing and packaging technologies.

Evaluation of the antioxidant and antimicrobial activity of common species of seaweeds from the west coast of Ireland in order to produce new functional foods, would therefore provide valuable information which could be utilised in the development of new functional food products from an unexploited natural resource. Such products could also potentially increase consumption of seaweed in a non-seaweed consuming population thus creating new value added food products. The development of such functional foods opens up new possibilities for seaweeds. Incorporation of seaweeds into existing products such as convenience meat products offers new opportunities for such an industry to improve their “image” while addressing consumer demands.

CHAPTER 3

MATERIALS AND METHODS

An explanation of experimental methodology employed throughout the thesis

3.1 Raw material

Laminaria digitata, *Laminaria saccharina*, *Himanthalia elongata* (Phaeophyta), *Palmaria palmata*, *Chondrus crispus* (Rhodophyta) and *Enteromorpha spirulina* (Chlorophyta) were purchased from Quality Sea Veg., Co Donegal, Ireland (Fig. 3.1).

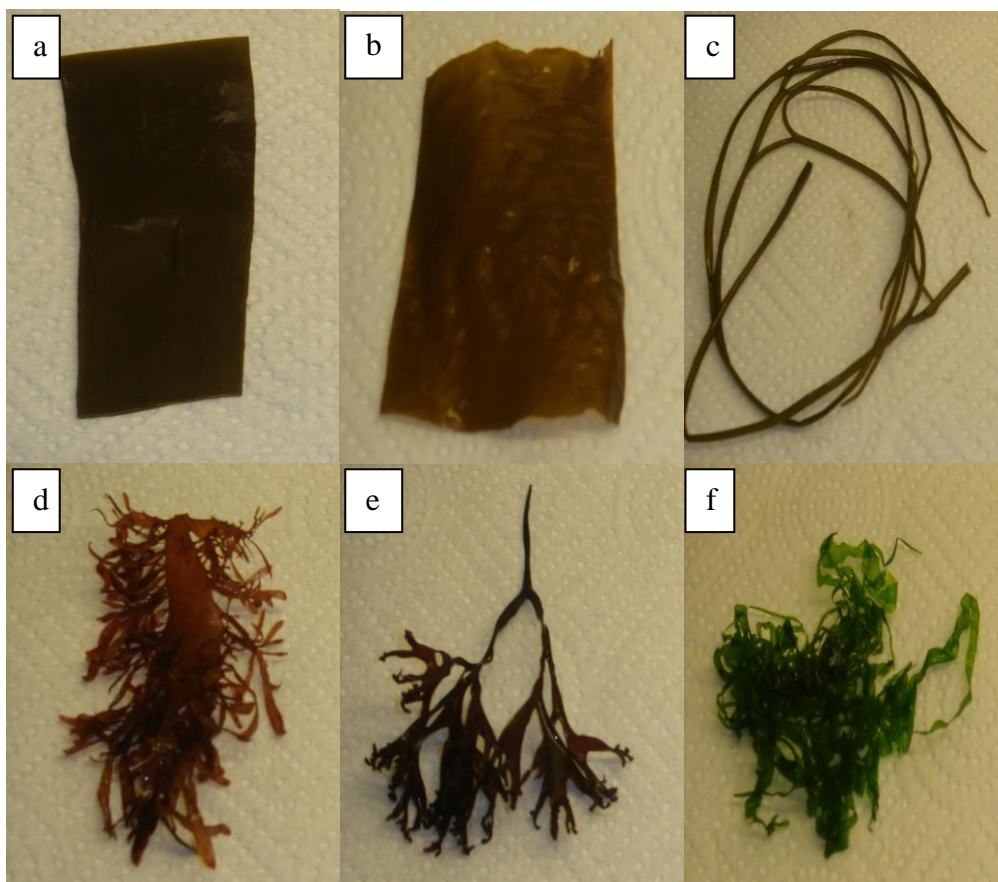


Fig. 3.1 Picture of (a) *L. digitata*, (b) *L. saccharina*, (c) *H. elongata*, (d) *P. palmata*, (e) *C. crispus* and (f) *E. spirulina*

Seaweed samples studied in this thesis were collected in June and September 2008, January and February 2010, and March, August and October 2011. The seaweeds were washed thoroughly with freshwater to remove epiphytes and salt, and stored at

4 °C until analysis. An overview of the experiments carried out in this thesis can be seen in Fig. 3.2.

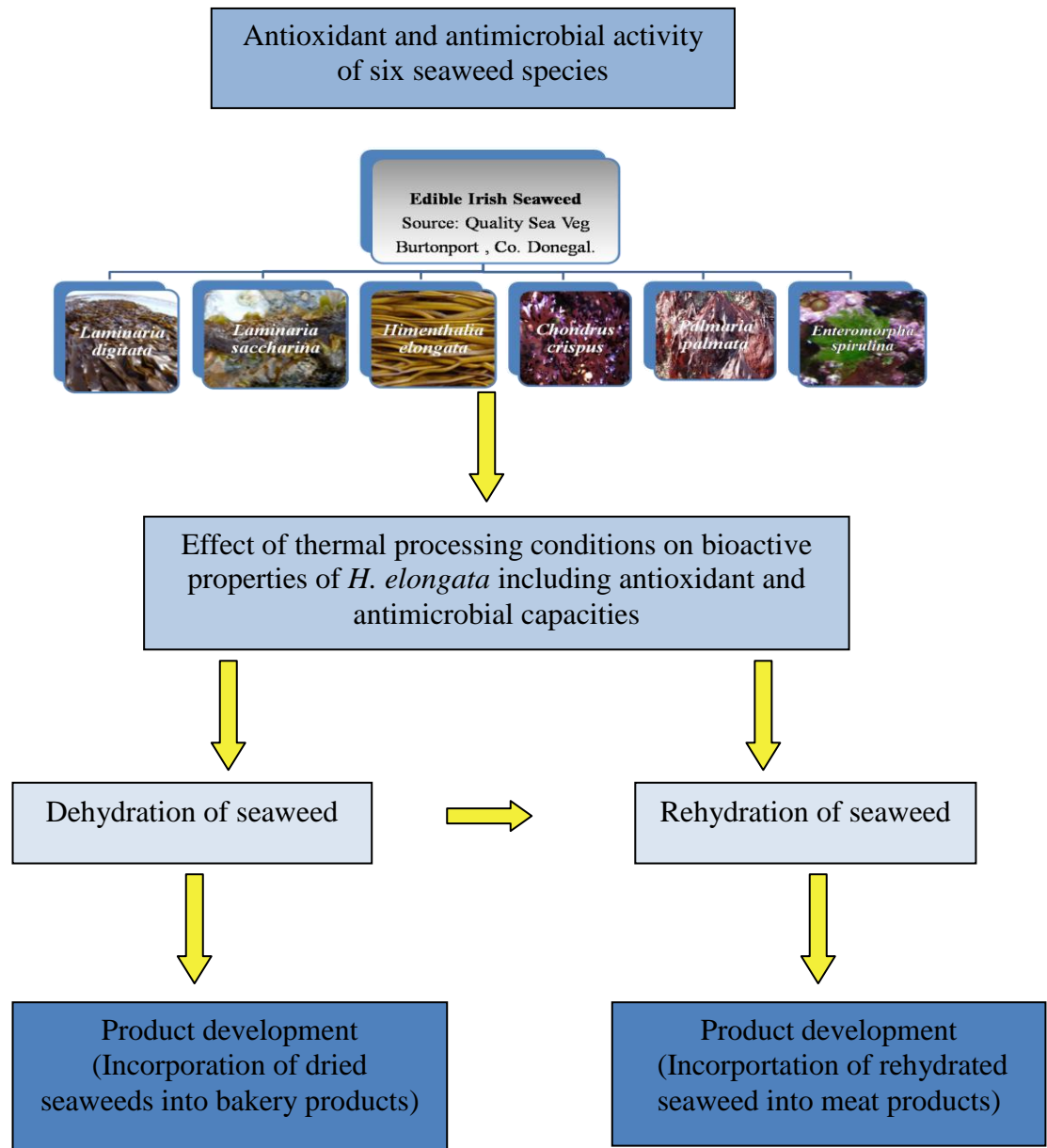


Fig. 3.2 Overview of the experiments carried out in the thesis

3.2 Extraction of phytochemicals

Frozen seaweed samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50 ml of methanol (60%) under nitrogen atmosphere for 2 hours (h). The extraction was carried out at 40 °C at 100 rpm in a shaker incubator (Innova 42, Mason Technology, Dublin, Ireland). Samples were filtered and centrifuged at 10,000 rpm for 15 min (Sigma 2K15, Mason Technology, Dublin, Ireland). Resulting extracts were evaporated to dryness using vacuum polyevaporator (Buchi Syncore Polyvap, Mason Technology, Ireland) at 60 °C. A pressure gradient program was designed for evaporation of the solvents with vacuum conditions of, 337 and 72 millibar (mbar) for methanol and water, respectively.

3.3 Preparation of seaweed extract stock solutions

Stock solutions of seaweed extracts were prepared for analysis by dissolving 1000 microgram (µg) of dried seaweed extract in 1 ml of deionised water. For antimicrobial analysis dried seaweed extracts were dissolved in Tryptic Soy Broth (TSB).

3.4 Total phenolic content

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Taga *et al.*, 1984). In this procedure, 100 µl aliquot of stock sample (extract concentration 1000 µg/ml of water) was mixed with 2.0 ml of 2% Na₂CO₃ and allowed to stand for 2 min at room temperature. Then 100 microlitres (µl) of 50% Folin-Ciocalteu's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nanometre (nm) using spectrophotometer (Milton Roy Spectronic 1201). The total phenolic contents of the

samples were expressed as mg gallic acid equivalent per gram of extract (mg GAE/g).

3.5 Total flavonoid content

Total flavonoid content (TFC) was determined according to the method of Zhishen *et al.* (1999). Briefly, a 250 μ l aliquot of each extract was mixed with 1.25 ml of double distilled (dd) H₂O and 75 μ l of 5% NaNO₂ solution. After 6 min, 150 μ l of 10% AlCl₃.H₂O solution was added. After 5 min, 0.5 ml of 1 molar (M) NaOH solution was added and then the total volume was made up to 2.5 ml with dd H₂O. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. Quercetin was used to prepare the standard curve and results were expressed as mg Quercetin equivalents (QE)/g extract.

3.6 Total condensed tannin content

Total condensed tannin content (TTC) was determined according to the method of Julkunen-Titto (1985). Briefly, a 50 μ l aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol) and then 750 μ l of concentrated HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. The absorbance against blank was read at 500 nm. (+)-catechin was used to prepare the standard curve and results were expressed as mg catechin equivalents (CE)/g extract.

3.7 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Yen and Chen (1995) with some modifications.

Briefly, a 100 µl aliquot of test sample was placed in a 96-well microtitre plate and 100 µl of 0.16 millimolar (mM) DPPH methanolic solution was added. The mixture was shaken and incubated for 30 min in darkness at 25 °C. Changes in the absorbance of the samples were measured at 517 nm using a microplate reader (Powerwave, Biotek, VT, USA). Ascorbic acid (AscA) used as a positive control.

The ability to scavenge the DPPH radical was calculated using the following equation given by Duan *et al.* (2006):

$$\text{Scavenging effect (\%)} = \left[1 - \left(\frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}} \right) \right] \times 100 \quad \text{Eq. 3.1}$$

Where; A_{control} is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{\text{sample blank}}$ is the absorbance of the sample only (sample without any DPPH solution). DPPH results were interpreted as % scavenging or the “efficient concentration” or EC_{50} value which is the concentration of substrate that causes 50% loss of the DPPH activity.

3.8 Antimicrobial activity

3.8.1 Microbial culture

Two species of common food pathogenic and two species of food spoilage bacteria selected for this study were *Listeria monocytogenes* ATCC 19115, *Salmonella abony* NCTC 6017, *Enterococcus faecalis* ATCC 7080 and *Pseudomonas aeruginosa* ATCC 27853, respectively (Medical Supply Company, Dublin, Ireland). All cultures

were maintained at -70 °C in 20% glycerol stocks and grown in Tryptic Soy Broth (TSB) at 37 °C; apart from *P. aeruginosa* which was incubated at 30 °C to obtain sub-cultures. Working cultures were prepared from sub-cultures and grown at optimal conditions for each bacterium for 18 h before analysis. Bacterial suspensions were then prepared in saline solution (NaCl 0.85%, BioMérieux, Marcy L'Etoile, France) equivalent to a McFarland standard of 0.5, using the Densimat photometer (BioMérieux Inc, France) to obtain a concentration of 1×10^8 colony forming units (CFU)/ml. This suspension was then diluted in TSB to obtain a working concentration of 1×10^6 CFU/ml.

3.8.2 Antimicrobial activity assay

The influence of varying concentrations of extract on efficacy were assessed against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* using 96-well microtitre plates (Sarstedt Ltd., Bath, United Kingdom). Extracts achieved from 5 g of fresh seaweed were dissolved in 2.5 ml of TSB and 200 µl was added to the first row of each plate. All other wells were filled with 100 µl of TSB and 100 µl from the first well was serial diluted two-folds along each column. Finally, 100 µl of bacterial suspension containing 1×10^6 CFU/ml was added to the wells. The last column was used for bacterium and media controls and sample blanks were prepared for all of the extracts. Absorbance readings were then taken at 0 and 24 h at 600 nm using a microplate spectrophotometer (Powerwave, Biotek) with 20 seconds agitation before each optical density (OD) measurement. Analysis of growth over time was also performed on most effective extracts. OD measurements were taken every three hours for 24 h. Sodium benzoate and sodium nitrite were used as controls. Percentage inhibition was calculated as follows:

$$\text{Bacterial inhibition (\%)} = \left[\left(\frac{O - E}{O} \right) \right] \times 100 \quad \text{Eq. 3.2}$$

Where; O is (OD of the Organism at 24 h - OD of the Organism at 0 h) and E is (OD of the Extract at 24 h – Blank at 24 h) – (OD of the Extract at 0 h- Blank at 0 h).

Results were interpreted by categorising percentage inhibitions based on inhibition intensity as given in Table 3.1.

Table 3.1 Categories of growth inhibition in antimicrobial assays

Classification criteria	Classes
Inhibition (%)	Inhibition intensity
100	Very strong
90-100	Strong
50-90	Moderate
<50	Weak

Source: Dubber and Harder (2008)

3.9 Texture evaluation

Shear tests were performed using an Instron Universal Testing Machine (Model 4301, Canton MA, USA) supported with Bluehill 2 version 2.14 analysis software for materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminium plate with dimensions of 10 x 6 cm², thickness 1.3 cm and with an

opening of 3 mm in the centre was supported in the Instron base. Seaweed samples (5 g) were sheared at a speed of 200 mm/min. The cutting implement was allowed to travel the depth of the seaweed, cutting through the sample and seaweed hardness was defined as the peak of force-deformation curve recorded in Newtons per mm (N/mm).

3.10 Colour measurement

The colour of samples was measured using a colourimeter (CIE Lab ColourQuest XE, Hunter Associates, Reston, VA, USA). The colourimeter was calibrated against a standard white reference tile ($L^* = 93.97$; $a^* = -0.08$ and $b^* = 1.21$). The colour values were represented on the CIE colour scales in terms of L^* (lightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness). From these values, total colour change from fresh (DE) was calculated according to the following equation:

$$DE = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad \text{Eq. 3.3}$$

Where; L^*_0 , a^*_0 and b^*_0 are the readings at time zero and L^* , a^* and b^* are the individual readings at each drying time.

3.11 Total Dietary Fibre

Total dietary fibre (TDF) was determined by Sigma analysis kit (Sigma-Aldrich, Inc., USA) based on AOAC method 991.43. Samples were cooked at 100 °C with heat stable α -amylase to initiate gelatinization, hydrolysis and depolymerisation of starch. The samples were incubated at 60 °C with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to

glucose). The samples were then treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerised protein and glucose. The residue was filtered, washed, dried and weighed. One duplicate was analysed for protein and the other was incubated at 525 °C to determine ash. The TDF was determined as the weight of the filtered and dried residue less the weight of the protein and ash.

3.12 Processing conditions applied to *H. elongata*

3.12.1 Dehydration of *H. elongata*

Fresh seaweeds were washed and cut manually with stainless steel knife into rectangular samples of approximately $3 \times 0.5 \times 0.2$ cm. Five grams of sample were placed on a flat tray and dried in a hot air oven (Innova 42, Mason Technology, Ireland) at different temperatures of 25, 30, 35 and 40 °C. The air velocity was set at 2.0 ± 0.1 m/s as measured with VWR Enviro-meter digital anemometer (VWR, Ireland). Samples were withdrawn after every hour until 8 hours and then after every 8 h for 24 h. The dry solids content was determined by employing control samples using an oven at 105 °C until constant weight of the sample was attained. The relative humidity was monitored with a data logger (Grant 1001, United Kingdom).

3.12.2 Rehydration/Hydrothermal processing of *H. elongata*

Dried or semi-dried seaweed samples (original 5 g samples) were rehydrated by immersion in 2 L of distilled water kept at the specified rehydration temperatures (20, 40, 60, 80 and 100 °C) using a water bath (Lauda, Aqualine AL5, Mason Technology, Ireland). Samples were removed every 5 min until a constant weight

was achieved. After rehydration, the seaweeds were drained using a wire mesh strainer and placed on ice to cool before the extraction procedure.

3.12.3 Steaming of *H. elongata*

Regular steaming was performed on dried and fresh seaweeds using an atmospheric steam cooker (Kenwood, FS360, Havant, United Kingdom) at 100 °C. The seaweed samples (5 g), were placed in the centre tray of the steam cooker, covered with the lid and steamed over 2 L of boiling water for 45 and 50 mins. After the steaming process, the processed seaweeds were drained and placed on ice to cool before the extraction procedure.

3.12.4 Microwaving of *H. elongata*

Fresh seaweed samples (5 g) were placed in a pyrex bowl, covered with a plastic film to prevent water loss and microwaved in a domestic microwave oven (Sharp Platinum Collection, Uxbridge, R-957, United Kingdom) at 450 and 900 watts (W) for 30 and 20 seconds (s), respectively. After microwaving, the seaweeds placed on ice to cool before the extraction procedure.

3.13 Modelling dehydration and rehydration kinetics of *H. elongata*

3.13.1 Drying kinetics expressed in terms of empirical models

The data obtained experimentally for the four different temperatures studied (25, 30, 35 and 40 °C) was plotted in the form of the dimensionless variable moisture ratio (MR) versus time (expressed in hours):

$$\text{Moisture ratio (MR)} = \frac{W - W_e}{W_0 - W_e} \quad \text{Eq. 3.4}$$

Where; W is the moisture content at any time t , W_e the equilibrium moisture content and W_0 is the initial moisture content and all expressed as dry basis (g water/g dry solids). The experimental sets (MR Vs time, t) were fitted to three different empirical models from the literature (Newton, Logarithmic and Henderson-Pabis), using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). The model equations are as follows:

$$\text{Newton: } MR = \exp(-kt) \quad \text{Eq. 3.5}$$

$$\text{Logarithmic: } MR = a \exp(-kt) + c \quad \text{Eq. 3.6}$$

$$\text{Henderson Pabis: } MR = a * \exp(-kt) \quad \text{Eq. 3.7}$$

3.13.2 Estimation of Diffusion co-efficient

The most widely studied theoretical model in thin layer drying of various foods is given by the solution of Fick's second law. The solution of Fick's second law for diffusion could be used to fit the experimental drying data. For sufficiently long drying times, the Fick's equation can be simplified into the following equation:

$$MR = \frac{8}{\pi^2} \left(e^{-D_{eff} \tau \left(\frac{\pi}{2l} \right)^2} \right) \quad \text{Eq. 3.8}$$

The above equation assumes that the effective diffusivity (D_{eff}) is constant and that shrinkage of the sample is negligible and can be further simplified into a straight line:

$$\ln(MR) = \ln \frac{8}{\pi^2} - D_{eff} \left(\frac{\pi}{2l} \right)^2 t \quad \text{Eq. 3.9}$$

Slope of the above line will give the value of effective diffusivity at different temperatures as:

$$\text{Slope} = -D_{eff} \left(\frac{\pi^2}{4l^2} \right) \quad \text{Eq. 3.10}$$

The effective diffusivity varies with the temperature according to an Arrhenius dependence of the type:

$$D_{eff} = D_0 \exp \left(- \frac{E_a}{R(T+273.15)} \right) \quad \text{Eq. 3.11}$$

Where; D_0 is diffusivity at an infinite temperature (m^2/s), E_a is the activation energy for moisture diffusion kilojoule per mole (kJ/mol), T is the drying temperature (Kelvin) and R is the gas constant (8.314 J/molK).

Upon linearization the slope indicates the activation energy:

$$\ln D_{eff} = \ln(D_0) + \left(- \frac{E_a}{R} \right) \frac{1}{T+273.15} \quad \text{Eq. 3.12}$$

3.13.3 Rehydration kinetics expressed in terms of empirical models

The data obtained experimentally for the five different temperatures studied (20, 40, 60, 80 and 100 °C) were plotted in the form of the dimensionless variable rehydration ratio (RR) versus time (expressed in min). RR was calculated as follows:

$$\text{Rehydration ratio (RR)} = \frac{W - W_e}{W_o - W_e} \quad \text{Eq. 3.13}$$

Where; W is the moisture content at any time t , W_e the equilibrium moisture content and W_o is the initial moisture content and all expressed as dry basis (g water/g dry solids).

The experimental sets (RR Vs time, t) were fitted to four different empirical models from the literature (Weibull, Peleg, First Order Rehydration Kinetics Model, Exponential Association Model), using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). In order to prove the temperature dependence of the rate constant (k), the Arrhenius equation was applied, graphically representing $\ln k$ versus $1/T$ (Simal *et al.*, 2005). The model equations are as follows:

$$\text{Weibull: } RR = W_e + (W_o - W_e) \exp \left[-\left(\frac{t}{\beta}\right)^\alpha \right] \quad \text{Eq. 3.14}$$

$$\text{Peleg: } RR = W_e + \frac{t}{k_1 + k_2 t} \quad \text{Eq. 3.15}$$

$$\text{First Order Rehydration Kinetics: } RR = W_e + (W_o - W_e) \exp(-k_R t) \quad \text{Eq. 3.16}$$

$$\text{Exponential Association Model: } RR = W_e [1 - \exp(-k_R t)] \quad \text{Eq. 3.17}$$

3.13.4 Dehydration and rehydration texture kinetics expressed in terms of empirical models

The zero and first-order equations were used to describe the texture changes in *H. elongata* over the dehydration and rehydration process:

$$\text{Zero - order: } C = C_0 \pm kt \quad \text{Eq. 3.18}$$

$$\text{First - order: } C = C_0 \exp(-kt) \quad \text{Eq. 3.19}$$

Where; C is the quality factor, C_0 is the initial value of the quality factor and k is the rate constant.

3.14 Response surface methodology (RSM) experimental design

In order to investigate the effect of factors (rehydration/hydrothermal processing time and temperature or seaweed and white flour concentrations) on the phytochemical constituents of *H. elongata* or breadsticks in Chapters 7 and 8, a central composite design with two factors was performed. RSM is a suite of mathematical and statistical techniques used to search for optimum conditions of factors for desirable responses, and evaluating the relative significance of several treatment factors even in the presence of complex interactions. The design leads to the generation of contour plots by linear or quadratic effects of the key variables and a model equation is derived that fits the experimental data to calculate the optimal

response of the system and cuts down on the number of experiments required (Liyana-Parthirana and Shahidi, 2005; Zhang *et al.*, 2007 and Prasad *et al.*, 2011).

The central composite design was applied using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA, USA). The total number of experiments generated from the software with two factors was 10 ($= 2^k + 2k + 2$), where k is the number of factors. Eight experiments were augmented with two replications at the centre points. The independent variables were blanching time (10 - 30 min) and blanching temperature (60 - 90 °C) which are the factors that have the most significant effect on the seaweed phytochemicals during the blanching process. Experimental data from the central composite design was analysed and fitted to a polynomial regression model below:

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_1 \chi_1^2 + \beta_2 \chi_2^2 + \beta_{12} \chi_1 \chi_2 \quad \text{Eq. 3.20}$$

Where; Y is response calculated by the model: β_0 is a constant, β_1 , β_2 and β_{12} are linear, squared and interaction coefficients, respectively.

The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (F -value) obtained from the analysis of variance (ANOVA) that was generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level ($\alpha = 0.05$). Three-dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables). The multi-response analysis of the response surface

design using desirability approach was used to optimise the experimental variables. The desirability function is an approach for solving the optimization problem of several responses and is applied when various responses have to be considered at the same time and it is necessary to find optimal compromises between the total numbers of responses taken into account. This methodology is based on first constructing a desirability function for each individual response, and then it is possible to obtain the overall desirability.

3.15 Scanning electron microscopy

The structure of the seaweed samples (fresh, dried and rehydrated) was studied using scanning electron microscope (Hitachi SU6600, Hitachi High-Technologies Europe GmbH, Germany). The samples were first ground into fine powder form and then suspended in ethanol to obtain a 1% suspension. The suspension was sprinkled on double stick tape fixed on an aluminium stub, and the powdered seaweed was coated with gold: palladium (60:40). An accelerating potential of 5 kilovolts (kV) was used during micrography.

3.16 Incorporation of seaweeds into bakery products

Seaweed and flour blends were prepared by replacement method according to the RSM experiment. The percentages of seaweed and white flour from the RSM were based on percentages of overall flour in the preparation formula (flour consisted of 60.79% of the preparation formula), with the remaining percentage of flour comprising of wholemeal. In this work section, the bakery product developed was a breadstick. To prepare the breadsticks, firstly, the yeast was dissolved in the water and added to the dry ingredients (except seaweed). The ingredients were mixed at

slow speed for 2 min, then at medium speed for 4 min (Hobard A120 mixer, Hobard MFG Co. Ltd, London, UK). Seaweed was then added and mixed again for a further 2 min. The dough was placed on trays and left to develop for 45 min then moulded into breadstick shapes by hand and proofed in a proofer (Sveba Dahlen, Sveba Dahlen, Fristan, Sweden) at 33 °C, 78% RH for 40 min. The breadsticks were then baked in an oven (Sveba Dahlen, DC 44, Sveba Dahlen, Fristan, Sweden) at 210 °C for 20 min with 10 seconds of steam at the beginning.

3.17 Incorporation of seaweeds into meat products

In this work section, the meat product developed constituted cooked beef patties. Five different patty formulations were prepared containing 0, 10, 20, 30 and 40% blanched seaweed. Lean beef ($\leq 5\%$ fat) was purchased from a local supermarket and stored immediately in a refrigerator at 4 °C. Meat was cut into smaller pieces using a sterile knife and ground in a meat grinder (Meteor MATR) which had been previously sterilised and chilled (4 °C). The seaweed was added to each of the mixtures in sterile bowls and mixed by hand with sterile utensils until the seaweed was homogenous throughout the meat. The final temperature of the meat was < 12 °C in all cases and patties were formed using a manual circular shaped mould. The patties were 1 cm thick and weighed 50 ± 0.05 g and samples were cooked in an oven (Rational Combi-Dämpfer, Essex, United Kingdom) at 200 °C for 15 mins. It was ensured that the centre of the patties reached ≥ 70 °C for 2 minutes. The patties were then immediately cooled to 4 °C and placed in polyethylene bags (PA/PE, Broderick Bros. Ltd., Dublin, Ireland) and vacuum packed (La Minerva, Pack 16, Bologna, Italy). The samples were stored at 4 °C throughout the storage period (30 days) until analysis.

3.18 Cooking properties measurement

Patties were weighed before cooking and after chilling at 4 °C. To estimate the cooking yield, their weights were expressed as a percentage of the initial weight using the following calculation:

$$\text{Cooking yield (\%)} = 100 \times \frac{\text{cooked weight (g)}}{\text{raw weight (g)}} \quad \text{Eq. 3.21}$$

3.19 Bacterial enumeration

Each patty sample (25 g) was taken aseptically and placed in a sterile stomacher bag with 225 ml of peptone water (Scharlau Chemie, Barcelona, Spain). After 2 min in a stomacher blender (Make and model), appropriate decimal dilutions were spread-plated (100 µl) onto Plate Count Agar (PCA) (Scharlau Chemie, Barcelona, Spain) for total viable counts and incubated at 37 °C for 24 h. The results were expressed as logarithms of colony forming units per gram of sample (log CFU/g). Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

3.20 pH measurement

The pH of patties (10 g homogenised in 50 ml distilled water) was determined using an Orion Model 520A pH metre (AGB Scientific Ltd) throughout the storage period. Three readings were taken for each sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

3.21 Lipid oxidation measurement

Lipid oxidation was assessed on the basis of the amount of malondialdehyde formed during storage. Malondialdehyde is the end-product of lipid peroxidation and was

evaluated using the TBARS assay with some modifications (Oussalah *et al.*, 2006). A 10 g portion of each meat sample was blended with 50 ml of distilled deionised water and 10 ml of 15% trichloroacetic acid (TCA) in a stomacher blender (Stomacher 400, Seward Medical, England) for 2 min at 260 rpm. The homogenate was centrifuged at 1500 gravity for 5 min and the supernatant fluid was filtered through a Durapore 0.45 µm HV membrane filter (Millipore). A 2 ml aliquot of 60 mmol/L TBA reagent was added to 8 ml of the clear filtrate and vortexed for 15 s and then heated in a boiling water bath for 10 min to develop a pink colour. After cooling on ice to ambient temperature (~ 20 °C), the absorbance of the supernatant was measured spectrophotometrically at 532 nm (Milton Roy Spectronic 1201). The concentration of malondialdehyde in analysed samples was calculated on the basis of a standard curve obtained using serial dilutions of 1,1,3,3-tetramethoxypropane solution. The TBARS value was expressed as mg malondialdehyde/kg (mg MDA/kg) of sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

3.22 Sensory characteristics

The sensory acceptance test was conducted in a standardised sensory test room (ISO 8589, 2007). Untrained panelists ($n = 20$) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (20 g) were served on white paper plates with random three-digit numbers and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall acceptability of the product (maximum of 5), where 5 was “like extremely” and 1 was “dislike

extremely”. The overall quality (maximum of 25) was computed by combining scores of all five attributes.

3.23 Statistical analysis

All experiments were performed in duplicate and replicated at least three times. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA, USA). Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $P < 0.05$.

The goodness of fit of the tested dehydration mathematical models to the experimental data was evaluated from the coefficient of determination (R^2), Sum square error (SSE), root mean square error (RMSE) and the chi-square (χ^2) between the predicted and experimental values. The higher the R^2 values and the lower the RMSE, SSE and χ^2 values, the better is the goodness of fit. The reduced chi-square can be calculated as follows:

$$SSE = \frac{1}{N} \sum_{i=1}^N (MR_{exp} - MR_{pred})^2 \quad \text{Eq. 3.22}$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (MR_{exp} - MR_{pred})^2} \quad \text{Eq. 3.23}$$

$$N^2 = \frac{\sum_{i=1}^N (MR_{exp,i} - MR_{pred,i})^2}{N - z} \quad \text{Eq. 3.24}$$

Where; $MR_{exp,i}$ is the experimental moisture ratio, $MR_{pred,i}$ is the predicted moisture ratio, N is the number of observations and z is the number of constants.

For rehydration models the equations were as follows:

$$SSE = \frac{1}{N} \sum_{i=1}^N (RR_{exp} - RR_{pred})^2 \quad \text{Eq. 3.25}$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (RR_{exp} - RR_{pred})^2} \quad \text{Eq. 3.26}$$

$$R^2 = \frac{\sum_{i=1}^N (RR_{exp,i} - RR_{pred,i})^2}{N - z} \quad \text{Eq. 3.27}$$

Where; $RR_{exp,i}$ is the experimental rehydration ratio, $RR_{pred,i}$ is the predicted rehydration ratio, N is the number of observations and z is the number of constants.

CHAPTER 4

INVESTIGATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY FROM SIX SPECIES OF EDIBLE IRISH SEAWEEDS

*An assessment of the antioxidant and antimicrobial activity of six species of edible
Irish seaweeds (Laminaria digitata, Laminaria saccharina, Himanthalia elongata,
Palmaria palmata, Chondrus crispus and Enteromorpha spirulina)*

*The results from this chapter were published as a peer-reviewed article in the
International Food Research Journal*

Summary

The following chapter involved a screening of six species of edible Irish seaweeds; *Laminaria digitata*, *Laminaria saccharina*, *Himanthalia elongata*, *Palmaria palmata*, *Chondrus crispus* and *Enteromorpha spirulina* for potential bioactivity. Extraction of secondary metabolites was carried out using different solvents (methanol, ethanol and acetone) to determine antioxidant and antimicrobial properties of the dried extracts. The total phenolic contents (TPC) of the dried methanolic extracts of the different seaweeds were significantly different ($P < 0.05$). Methanolic extracts of *H. elongata* exhibited the highest TPC at 151.3 mg GAE/g of seaweed extract and also had the highest DPPH scavenging activity ($P < 0.05$) with a 50% inhibition (EC_{50}) level at 0.125 $\mu\text{g}/\text{ml}$ of extract. *H. elongata* also had the highest total tannin content (TTC) and total flavonoid contents (TFC) ($P < 0.05$) of 38.34 mg CE/g and 42.5 mg QE/g, respectively. Antimicrobial activity was determined using a microtitre method which allowed detection of bacterial growth inhibition at low levels. All methanolic seaweed extracts inhibited the food spoilage and food pathogenic bacteria tested; *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*, except *C. crispus* extracts. The dried methanolic extracts of red and green seaweeds had significantly lower antimicrobial activity than the brown species; *H. elongata* had the highest antimicrobial activity with up to 100% inhibition. The antimicrobial activity of red and green seaweed extracts significantly increased when ethanol and acetone were used as extraction solvents ($P < 0.05$). Inhibition of *E. faecalis* with *C. crispus* extracts increased from 39.28 to 100% when ethanol and acetone were applied as solvents.

4.1 Introduction

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Yuan *et al.*, 2005; Bansemir *et al.*, 2006; Chew *et al.*, 2008). The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidising agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds (Matsukawa *et al.*, 1997).

Reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxy radicals are formed in human cells by endogenous factors and result in extensive oxidative damage which can lead to age related degenerative conditions, cancer and a wide range of other human diseases (Reaven and Witzum, 1996; Aruoma, 1999). Phenolic compounds can act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Mohamed-Saiel, 2006). The term “phenolic compound” describes several hundred molecules found in edible plants that possess on their structure a benzene ring substituted by, at least, one hydroxyl group. Such compounds are commonly found in plants, including seaweeds (Manach *et al.*, 2004; Duan *et al.*, 2006). Polyphenols represent a diverse class of compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavononols, chalcones and flavan-3-ols), lignins, tocopherols, tannins and phenolic acids (Shukla *et al.*, 1997). Interest in new sources of natural

antioxidants and antimicrobials has increased in recent years in an attempt to reduce the use of synthetic forms such as BHA and BHT. Natural antioxidants from plant origin can react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration (Akoh and Min, 1997). Furthermore, antioxidants from natural sources can also increase the shelf life of foods. Therefore, the consumption of antioxidant and/or addition of antioxidant to food materials could protect the body as well as the foods against these events (Chandini *et al.*, 2008).

Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolyzable compounds. Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants (Haslam, 1989; Waterman and Mole, 1994). Phlorotannins are tannin compounds which have been found only in marine algae and not in terrestrial plants. Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga (Ragan and Glombitza, 1986; Waterman and Mole, 1994; Arnold and Targett, 1998). Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (Ahn *et al.*, 2007). Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings (Hemat, 2007).

Flavonoids, the largest group of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical

scavenging properties (Kahkonen *et al.*, 1999). Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or UV radiation (Harbourne, 1994; Herrmann, 1989; Wallace and Fry, 1994).

Many marine plants, including seaweeds, often carry significantly less macro and microepibionts on their thalli compared to co-occurring biofilms on inanimate substrata (Hellio *et al.*, 2001; Lam and Harder, 2007). Therefore it has been assumed that seaweeds defend themselves against bacterial fouling by production of secondary metabolites that prevent attachment and growth of bacterial colonizers (Maximilien *et al.*, 1998).

Many researchers have reported on the antioxidant and antimicrobial activity of a range of seaweeds (Gonzalez del Val *et al.*, 2001; Ganesan *et al.*, 2008; Plaza *et al.*, 2009). However, reports on the antioxidant and antimicrobial activity of seaweed extracts from Ireland are very limited. With this in mind, the main aims of this chapter were as follows:

1. To investigate the antioxidant and antimicrobial activity of six common species of Irish seaweeds.
2. To determine extraction efficiencies of methanol, ethanol and acetone on antioxidant and antimicrobial activity.

4.2 Materials and methods

4.2.1 Raw material

Raw material is described in section 3.1. The *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina* samples studied in this chapter were collected in June and September 2008.

4.2.2 Preparation of dried seaweed extracts

Extraction procedure is described in section 3.2 with the following modifications: Seaweed samples (5 g) were extracted with 50 ml of either methanol, ethanol or acetone (60%) under nitrogen atmosphere for 2 hours. Resulting extracts were evaporated to dryness using vacuum polyevaporator (Buchi Syncore Polyvap, Mason Technology, Ireland) at 60 °C. A pressure gradient program was designed for evaporation of the solvents under vacuum conditions of 556, 337, 175 and 72 mbar for acetone, methanol, ethanol and water, respectively.

4.2.3 Preparation of seaweed extract stock solutions

Stock solutions of seaweed extracts were prepared as described in section 3.3.

4.2.4 Total phenolic content

The total phenolic concentration was measured as described in section 3.4.

4.2.5 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Yen and Chen (1995). Briefly, a 2.0 ml aliquot of test sample was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was shaken vigorously then left to stand at room temperature for 30 min in darkness. Changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer (Milton Roy Spectronic 1201). The ability to scavenge the DPPH radical was calculated using Eq. 3.1 in section 3.7.

4.2.6 Total flavonoid content

Total flavonoid content was determined as described in section 3.5.

4.2.7 Total condensed tannin content

Total condensed tannin content was determined as described in section 3.6.

4.2.8 Antimicrobial activity

4.2.8.1 Microbial culture

Microbial cultures were prepared as described in section 3.8.1.

4.2.8.2 Antimicrobial activity assay

The antimicrobial activity assays were carried out as described in section 3.8.2.

4.2.9 Statistical analysis

All experiments were performed in triplicate and replicated twice. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA) as described in section 3.23.

4.3 Results and Discussion

4.3.1 Total phenolic content of methanolic seaweed extracts

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007). Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties (Duan *et al.*, 2006; Kuda *et al.*, 2007; Wang *et al.*, 2009). The Folin-Ciocalteu method was applied to study the total phenolic content (TPC) of the seaweeds. Folin-Ciocalteu reagent determines total phenols, producing blue colour by reducing yellow heteropolyphosphomolybdate-tungstate anions (Athukorala *et al.*, 2006).

The TPC of dried methanol extracts of six Irish seaweeds as obtained in this study are presented in Table 4.1. The TPC of the studied seaweeds ranged from 37.66 to 151.33 mg GAE/g of extract. Extracts from brown seaweed, *H. elongata* the exhibited highest phenolic content; as compared to other brown species, *L. digitata* and *L. saccharina* which had TPC of 37.6 and 64.75 mg GAE/g extract, respectively. Red and green seaweeds contained less than half the phenolic content of *H. elongata*, but similar to that of the brown *L. digitata* species; *C. crispus*, *E. spirulina* and *P. palmata* contained 62.33, 49.75 and 42.83 mg GAE/g extract, respectively.

Table 4.1 Total phenolic content and antioxidant activity of methanolic extracts of *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina*

Species	Total phenolic content (mg GAE/g)	Antioxidant activity EC ₅₀ ^a
<i>L. digitata</i>	37.66±0.00 ^a	1.0±0.00 ^a
<i>L. saccharina</i>	66.75±3.72 ^b	10.0±0.16 ^b
<i>H. elongata</i>	151.33±6.75 ^c	0.125±0.14 ^c
<i>P. palmata</i>	42.83±3.26 ^d	25.0±0.06 ^d
<i>C. crispus</i>	62.33±1.04 ^b	5.0±0.04 ^e
<i>E. spirulina</i>	49.75±0.41 ^e	50.0±0.04 ^f
AscA	178.75±2.58 ^f	1.0±0.09 ^a

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-f) differ significantly ($P < 0.05$).

^arefers to µg/ ml of extract required to reduce DPPH radical by 50% (EC₅₀)

AscA refers to ascorbic acid

Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yukimoto, 2003). The TPC results of *H. elongata* and *L. saccharina* obtained in this study were higher than some reports for other brown seaweeds. Chandini *et al.* (2008) reported that brown seaweed extracts had a phenolic content of 24.61 and 49.16 mg GAE/g of seaweed extract. Ganesan *et al.* (2008) reported that crude methanolic extracts of red seaweeds had a yield of 1.5 to 4.1 mg GAE/g, which is lower than the phenolic contents of the red species studied in this chapter.

Wang *et al.* (2009) reported the TPC in different Icelandic seaweeds to be ranging from 4 to 242 mg phloroglucinol equivalents (PGE)/g extract; *P. palmata* had the lowest total phenolic content of the seaweeds tested, which is in agreement with the results obtained for red seaweeds in the present study. However, the total phenolic

content of *P. palmata* was over ten times lower in the Icelandic seaweeds which could be due to differences such as seasonality or maturity of the plant (Wang *et al.*, 2009). The results of the present study are promising as algal polyphenolic compounds are effective antioxidants in delaying oil rancidity, therefore the seaweed extracts could have potential in food applications (Yan *et al.*, 1996).

4.3.2 DPPH radical scavenging activity of methanolic seaweed extracts

DPPH reagent has been used extensively for investigating the free radical scavenging activities of various extracts. In the DPPH test, the dried extracts are potentially able to reduce the stable DPPH radical to the yellow coloured diphenylpicrylhydrazine. The assay is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Shon *et al.*, 2003). DPPH results are often interpreted as the “efficient concentration” or EC₅₀ value, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (Molyneux, 2004).

The EC₅₀ values of DPPH radical scavenging activity from dried methanolic extracts of seaweeds are presented in Table 4.1. The antioxidant activities of all six seaweeds were significantly different ($P < 0.05$), and EC₅₀ levels ranged from 0.125 to 50 µg/ml. Brown seaweed, *H. elongata* showed the highest activity with 50% inhibition of DPPH radical at a concentration as low as 0.125 µg/ml of extract. Other brown seaweeds also showed good inhibition at EC₅₀ levels of 1 and 10 µg/ml of extract (*L. digitata* and *L. saccharina*, respectively). Red and green seaweeds, *P. palmata* and *E. spirulina* had significantly lower antioxidant activity with EC₅₀ levels at 25 and 50

µg/ml, respectively ($P < 0.05$). *C. crispus* had an EC₅₀ level of 5 µg/ml which was the highest among the red seaweed species studied in this work.

The present results are in line with Wang *et al.* (2009) and Yan *et al.* (1999), who also found that brown algae contained higher amounts of polyphenols and DPPH radical scavenging activity than red and green algae. However, Chandini *et al.* (2008) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range of 17.79 to 23.16% at an extract concentration of 1000 µg/ml. Also, Duan *et al.* (2006) reported the DPPH radical scavenging activity of crude extract of red alga, *Polysiphonia urceolata* to be half that of the red seaweed species investigated in the present study. Wang *et al.* (2009) reported on the antioxidant activities of Icelandic seaweeds and found that brown species exhibited the most effective scavenging ability on DPPH radicals with an EC₅₀ of 10.7 g/ml. These results are substantially less effective than that of all six species of seaweeds in the present study which shows that Irish seaweeds have good potential as sources of natural antioxidants.

H. elongata had significantly more activity than the ascorbic acid control ($P < 0.05$) at the EC₅₀ level; however at higher concentrations the activity of ascorbic acid rapidly increased to 97.11% (10 µg/ml ascorbic acid). The ability of seaweed extracts to quench free radicals is known to take place over longer period of time than rapid acting synthetic antioxidants such as BHA. This may have benefits for extending the shelf life of processed foods during distribution and storage which may be a potential application for the extracts. The three brown seaweed extracts in this study had very effective DPPH radical scavenging activity as they had EC₅₀ values at

low levels (below 10 µg/ml) of extract. This would be beneficial for industrial application as low quantities of seaweed extracts could be utilised as effective antioxidants.

H. elongata had the best DPPH activity and also the highest total phenolic content. However, as previously discussed, *L. saccharina* had a significantly higher phenolic content than *L. digitata* ($P < 0.05$), but *L. digitata* had higher DPPH scavenging activity than *L. saccharina*. Also, *L. saccharina* and *C. crispus* had similar phenolic content ($P < 0.05$) yet there was a significant difference in their antioxidant activity. Brown seaweed *L. digitata* had a lower phenolic content than the red and green seaweeds, however it had significantly higher antioxidant activity ($P < 0.05$). This result implies that DPPH radical scavenging activity may not be directly related to total phenolic content which indicates the role of other compounds in the antioxidant activity of seaweeds. Antioxidant activity of marine algae may arise from pigments such as chlorophylls and carotenoids, vitamin and vitamin precursors including α -tocopherol, β -carotene, niacin, thiamine and ascorbic acid, phenolics such as polyphenolics and hydroquinones and flavonoids, phospholipids particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, which directly or indirectly contribute to the inhibition or suppression of oxidation processes (Shahidi, 2008).

4.3.3 Total flavonoids and total condensed tannins of methanolic seaweed extracts

The total flavonoid content (TFC) of the dried seaweed extracts are presented in Table 4.2. TFC in the studied seaweeds ranged from 7.66 to 42.5 mg QE/g of extract.

Brown species, *H. elongata* contained significantly higher TFC ($P < 0.05$) than the other brown seaweeds studied in this work. Red seaweeds, *P. palmata* and *C. crispus* had the lowest TFC at 6.83 and 7.41 mg QE/g extract, respectively ($P < 0.05$). Kahkonen *et al.* (1999) suggested that flavonoids are probably the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases (Ross and Kasum, 2002; Williams *et al.*, 2004).

Table 4.2 Total condensed tannins and total flavonoid content of methanolic extracts of *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina*

Species	Total condensed tannins (mg CE/g)	Total flavonoids (mg QE/g)
<i>L. digitata</i>	5.44±0.23 ^a	7.66±0.60 ^a
<i>L. saccharina</i>	6.17±0.32 ^b	9.66±0.25 ^b
<i>H. elongata</i>	38.34±0.91 ^c	42.50±0.86 ^c
<i>P. palmata</i>	3.19±0.35 ^d	6.83±0.25 ^d
<i>C. crispus</i>	4.35±0.55 ^e	7.41±0.49 ^{ad}
<i>E. spirulina</i>	3.21±0.50 ^d	19.05±0.73 ^e

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-e) differ significantly ($P < 0.05$).

Total condensed tannin content (TTC) of dried methanolic extracts of *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina* can be seen in Table 4.2. They ranged from 3.19 to 38.34 mg CE/g of extract. The content of tannins from the brown seaweed, *H. elongata* was significantly higher than the other

species ($P < 0.05$). Brown species *H. elongata*, *L. saccharina* and *L. digitata* contained 38.34, 6.17 and 5.44 mg CE/g extract, respectively. The red and green species contained lower TTC than the brown seaweeds, in the range of 3.19 to 4.35 mg CE/g of seaweed extract. Phlorotannins are a group of phenolic compounds which are restricted to polymers of phloroglucinol and have been identified from several brown algae. Many studies have shown that phlorotannins are the only phenolic group detected in brown algae (Jormalainen and Honkanen, 2004; Koivikko *et al.*, 2007).

Phlorotannin concentrations vary greatly among different species of brown seaweeds, as well as among different geographical areas. Concentration of phlorotannins are generally greater in furoid species than in kelps, which is in line with the results of the present study as *H. elongata* (furoid species) contained higher condensed tannins than the kelps, *L. digitata* and *L. saccharina* (Pavia and Toth, 2000). *H. elongata* was the most potent DPPH and hydroxyl radical scavenger, and also contained the highest levels of phenols, tannins and flavanoids. This suggests that algal polyphenols including tannins and flavonoids may be the principal constituents responsible for the antiradical properties of extracts from this species.

4.3.4 Antimicrobial activity of methanolic seaweed extracts

Food poisoning is a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety regulators and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. Nowadays, consumers are demanding foods which are fresh, natural and

minimally processed. Along with this, consumers are also concerned about the safety of foods containing synthetic preservatives. This has put pressure on the food industry and has fuelled research into the discovery of alternative natural antimicrobials (Shan *et al.*, 2007). In the majority of reports on antimicrobial activities of seaweed extracts, bacterial growth inhibiting activities were investigated by standard agar disc diffusion assays (Bansemir *et al.*, 2006; Kuda *et al.*, 2007; Shanmughapriya *et al.*, 2008). There have been few reports on quantitative methods being utilised for seaweed extracts such as the 96-well method which was applied in this study. Percentage inhibition of each seaweed extract was calculated over a 24 h period and the assays revealed different susceptibilities of the bacteria under investigation to the seaweed extracts.

The entire yield of dried extract from 5 g of each of the fresh seaweeds was dissolved in 2.5 ml TSB and utilised in the assay. The percentage inhibition of the highest concentration of methanolic seaweed extracts against food spoilage and food pathogenic bacteria are presented in Table 4.3.

Table 4.3. Percentage inhibition of most effective concentrations of methanolic extracts of *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina* against selected food pathogenic ^(a) and food spoilage ^(b) bacteria

Species	<i>L. monocytogenes</i> ^a	<i>S. abony</i> ^a	<i>E. faecalis</i> ^b	<i>P. aeruginosa</i> ^b
<i>L. digitata</i>	100.00±00 ^a	86.36±3.50 ^a	75.35±3.65 ^a	72.16±2.34 ^a
<i>L. saccharina</i>	93.49±2.00 ^b	98.16±2.07 ^b	97.07±2.67 ^{bc}	93.66±3.84 ^b
<i>H. elongata</i>	100.00±0.00 ^a	100.00±0.00 ^b	100.00±0.00 ^c	98.40±2.49 ^c
<i>P. palmata</i>	62.09±4.11 ^c	2.21±0.87 ^c	39.28±4.36 ^d	19.22±3.60 ^d
<i>C. crispus</i>	-3.88±1.36 ^d	-10.70±0.96 ^d	-66.08±3.89 ^e	-31.72±1.52 ^e
<i>E. spirulina</i>	44.72±1.77 ^e	21.21±3.69 ^e	24.13±1.18 ^f	12.45±3.72 ^f
Sodium benzoate	96.55±4.07 ^{ab}	97.34±3.07 ^b	89.42±3.72 ^g	99.04±0.58 ^c
Sodium nitrite	96.19±2.93 ^{ab}	98.45±2.26 ^b	93.75±2.16 ^{gb}	97.27±2.69 ^{bc}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-g) differ significantly (*P* < 0.05).

Generally, all of the common food spoilage and food pathogenic bacteria under investigation (*L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa*) were susceptible to the seaweed extracts, causing substantial growth inhibition of the test strains, with the exception of *C. crispus* extracts which enhanced the growth. The entire spectrum of inhibitory effects is reported as outlined in Table 3.1 as seen in section 3.8.2.

At the highest dried extract concentrations (8, 6.4, 6.2, 5, 4.8 and 5.8 mg/ ml for *L. digitata*, *H. elongata*, *L. saccharina*, *P. palmata*, *C. crispus* and *E. spirulina*, respectively), all of the brown seaweeds had above 70% inhibition. *H. elongata* had the highest antimicrobial activity showing strong to very strong activity at 6.4 mg/ ml of extract against *L. monocytogenes*, *S. abony* and *E. faecalis*, and *P. aeruginosa* (100, 100, 100 and 98.4%, respectively). This was followed by *L. saccharina* which

had strong to very strong activity against *S. abony*, *E. faecalis*, *P. aeruginosa* and *L. monocytogenes* (98.16, 97.07, 93.66 and 93.49%, respectively). *L. digitata* displayed very strong activity against *L. monocytogenes* (100%) and moderate activity against *S. abony*, *E. faecalis* and *P. aeruginosa* (86.36, 75.35 and 72.16%, respectively).

In the case of antimicrobial activity against *L. monocytogenes*; there was no significant difference between the activity of brown seaweed extracts of *L. digitata* and *H. elongata*; however, *L. saccharina* had significantly lower activity ($P < 0.05$) compared to the other two brown seaweed species studied. When tested against *S. abony*, *E. faecalis* and *P. aeruginosa*; brown seaweeds, *H. elongata* and *L. saccharina* had significantly stronger activity than *L. digitata* ($P < 0.05$). The antimicrobial activities of the red and green seaweed extracts were significantly lower against the four strains of food spoilage and food pathogenic bacteria than the brown seaweed species ($P < 0.05$). *P. palmata* had moderate antimicrobial activity against *L. monocytogenes* (62.09%) and weak activity against *E. faecalis*, *P. aeruginosa* and *S. abony* (39.28, 19.22 and 2.21%, respectively). Methanolic extracts of *C. crispus* were the only seaweed extracts which had no antimicrobial activity against the four strains tested as it enhanced the growth of the food spoilage and food pathogenic bacteria. A possible explanation for this may be the presence of certain sugars and proteins in this seaweed species which could have enhanced the growth of the bacteria (Galland-Irmouli *et al.*, 1999). Green seaweed, *E. spirulina* had weak activity against *L. monocytogenes*, *E. faecalis*, *S. abony* and *P. aeruginosa* with inhibitions of 44.72, 24.13, 21.21 and 12.45%, respectively.

Sodium benzoate and sodium nitrite were utilised as controls; sodium benzoate is a common food preservative used in salad dressings, carbonated drinks and condiments. Sodium nitrite is a preservative used in meats and fish. According to the European Food Directive 95/2/EC on “Food Additives other than Colours and Sweeteners”, the maximum permitted level of sodium benzoate is 0.15 – 2 g/kg depending on the food product. Sodium nitrite is permitted at 0.3 g/kg in meat products. Sodium benzoate and sodium nitrite had similar bacterial growth inhibition to the brown seaweed extracts tested. This implies that the seaweed extracts examined in this study have activity in the same line as commercially applied antimicrobials.

Food spoilage due to the presence of bacteria causes economic losses on a global scale. Since many marine plants have evolved potent defence mechanisms against bacteria; there is interest in seaweeds as a source of natural antifouling and antimicrobial sources. In this context, the results of the present study show that extracts of brown seaweeds could have potential as a source for new antimicrobial agents equal to that of commercially applied synthetic antibacterial agents. Since the brown seaweeds were the most effective antimicrobials, significant differences between the different brown seaweed extract concentrations tested were found against food pathogenic and food spoilage bacteria and results are presented in Tables 4.4, 4.5 and 4.6.

Table 4.4 Percentage inhibition of methanolic extract of *L. digitata* against selected food pathogenic ^(a) and food spoilage ^(b) bacteria

Concentration (mg/ ml)	<i>L. monocytogenes</i> ^a	<i>S. abony</i> ^a	<i>E. faecalis</i> ^b	<i>P. aeruginosa</i> ^b
8.00	100.00±0.00 ^a	86.36±3.50 ^a	75.35±3.65 ^a	72.16±2.34 ^a
4.00	98.28±2.75 ^a	63.13±3.11 ^b	51.52±3.79 ^b	47.65±4.87 ^b
2.00	75.34±2.74 ^b	51.48±1.67 ^c	38.55±2.27 ^d	37.11±2.75 ^{cd}
1.00	66.29±3.74 ^c	48.02±1.88 ^c	43.94±2.98 ^{cd}	40.88±1.39 ^{bc}
0.50	62.29±3.90 ^c	45.94±4.22 ^c	45.33±3.15 ^{bc}	30.89±4.48 ^d
0.250	60.48±4.53 ^c	48.01±3.10 ^c	44.63±4.01 ^{cd}	35.13±3.74 ^{cd}
0.125	60.86±3.80 ^c	49.67±2.48 ^c	45.17±2.88 ^c	34.65±0.79 ^{cd}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-d) differ significantly ($P < 0.05$).

Table 4.5 Percentage inhibition of methanolic extract of *L. saccharina* against selected food pathogenic ^(a) and food spoilage ^(b) bacteria

Concentration (mg/ ml)	<i>L. monocytogenes</i> ^a	<i>S. abony</i> ^a	<i>E. faecalis</i> ^b	<i>P. aeruginosa</i> ^b
6.20	93.49±2.00 ^a	98.16±2.07 ^a	97.07±2.67 ^a	93.66±3.84 ^a
3.10	86.15±2.50 ^b	87.38±3.17 ^b	78.26±3.77 ^b	76.21±1.76 ^b
1.55	76.47±3.24 ^c	71.94±3.99 ^c	78.56±3.07 ^b	72.97±3.90 ^{bc}
0.775	74.66±2.38 ^c	77.13±2.05 ^d	81.63±3.60 ^b	77.12±3.97 ^{bc}
0.387	76.35±1.38 ^c	73.84±2.05 ^{cd}	86.38±2.81 ^c	77.10±2.19 ^{bc}
0.194	76.54±2.15 ^c	77.80±2.18 ^d	86.97±3.37 ^c	81.24±3.18 ^{cd}
0.097	76.17±2.48 ^c	77.48±1.80 ^d	88.49±2.77 ^c	82.31±2.22 ^d

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-d) differ significantly ($P < 0.05$).

Table 4.6 Percentage inhibition of methanolic extract of *H. elongata* against selected food pathogenic ^(a) and food spoilage ^(b) bacteria

Concentration (mg/ml)	<i>L. monocytogenes</i> ^a	<i>S. abony</i> ^a	<i>E. faecalis</i> ^b	<i>P. aeruginosa</i> ^b
6.40	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a	98.40±2.49 ^a
3.20	97.37±0.65 ^{ab}	95.40±1.42 ^a	88.41±2.13 ^b	91.16±1.52 ^a
1.60	96.14±2.26 ^{ab}	72.99±4.57 ^b	100.00±2.33 ^a	76.98±3.30 ^b
0.80	92.10±4.96 ^b	53.53±1.92 ^c	86.71±3.17 ^b	50.74±2.95 ^c
0.40	90.67±3.01 ^b	36.70±0.81 ^d	55.14±3.98 ^c	43.68±3.45 ^c
0.20	79.76±1.90 ^c	5.10±1.46 ^e	34.32±2.09 ^d	33.84±3.77 ^d
0.10	54.41±4.91 ^d	0.55±0.85 ^e	10.36±4.31 ^e	8.42±1.92 ^e

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-e) differ significantly ($P < 0.05$).

Percentage inhibition of *L. monocytogenes* was equally as effective when using 4 mg of *L. digitata* extract as double the concentration at 8 mg/ml ($P > 0.05$). However, the antimicrobial activities from 1 – 0.125 mg/ml extract were significantly lower ($P < 0.05$) achieving moderate activity (< 66.29% inhibition). When tested against *S. abony*, *E. faecalis* and *P. aeruginosa*; *L. digitata* extracts at concentration of 8 mg/ml were significantly higher than all other concentrations tested ($P < 0.05$) and had weak activity (< 50% inhibition at 1 mg/ml and lower). *L. saccharina* extracts (Table 4.5) exhibited strong inhibition activity at the highest concentration (6.20 mg/ml) against *S. abony*, *E. faecalis*, *P. aeruginosa* and *L. monocytogenes*, (98.16, 97.07, 93.66 and 93.49%, respectively). This was significantly higher than all other concentrations of extracts tested ($P < 0.05$).

However, even at the lowest concentration of extract tested (0.097 mg/ml) the activity against all tested bacteria was above 76%, which was stronger than that of

the other brown seaweeds. This could possibly be due to the high concentration of polysaccharides in *L. saccharina* which are known to have antimicrobial properties (Yamashita *et al.*, 2001). There was no significant difference ($P > 0.05$) in the activity of *H. elongata* extracts at concentrations of 6.4 and 3.2 mg/ml against the tested bacteria (Table 4.6). Starting from a concentration of 1.60 mg/ml and lower, there were significant differences in inhibition activity ($P < 0.05$). At concentrations of 0.4, 0.2 and 0.1 mg/ml; *H. elongata* extracts had weak activity against *S. abony*, *E. faecalis* and *P. aeruginosa* ($< 50\%$).

These results indicated that the antimicrobial activity of seaweed extracts were concentration dependant. At higher extract concentrations, *H. elongata* had the strongest activity ($P < 0.05$) compared to the other two brown seaweeds; however at lower concentrations, *L. saccharina* was most effective against the food spoilage and food pathogenic bacteria tested. This could possibly be due to differences in the composition of each of the extracts and the potency differences of such compounds at lower concentrations.

Analysis of the most effective concentration of extracts against food spoilage and food pathogenic bacteria was performed over time. Fig. 4.1 illustrates antimicrobial activity of the three brown seaweed extracts which were most effective over a 24 h period.

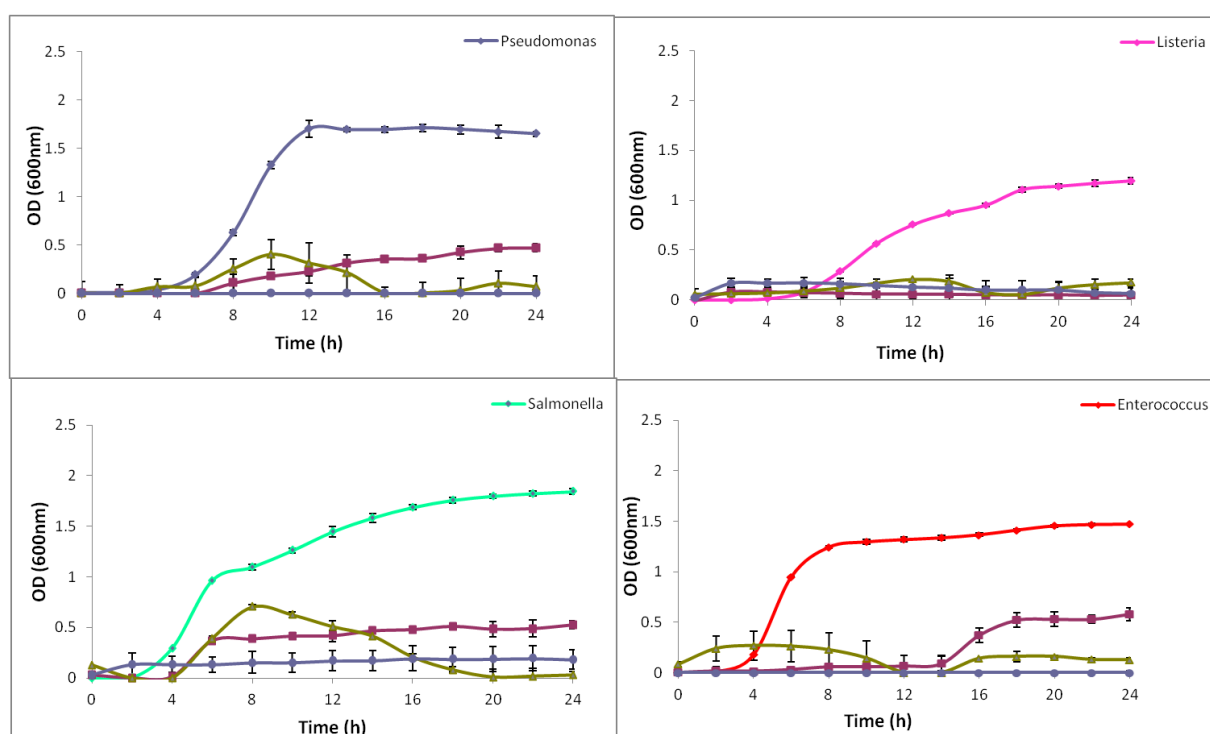


Fig. 4.1 Growth inhibition analysis of the highest effective concentration of brown seaweed extracts against common food spoilage and food pathogenic bacteria (■ : *L. digitata*; ■ : *L. saccharina*; ■ : *H. elongata*)

The seaweed extracts inhibited growth of each of the bacteria tested from the first hour resulting in lag phase extension. All three methanolic brown seaweed extracts displayed inhibition activity similar to that of the commercial controls. In previous reports investigating the effects of marine macroalgae extracts; antimicrobial compounds were detected in extracts of red and brown algae including *L. digitata* (Hellio *et al.*, 2001; Bansemir *et al.*, 2006; Dubber and Harder, 2008). *L. saccharina* and *H. elongata* have been less studied with regard to antimicrobial activity. Ely *et al.* (2004) analysed the antimicrobial activity of a brown seaweed *Sargassum marginatum* against two species of fish pathogenic bacteria and found its activity weak at 5 mg/ml. Comparing this to the results in the present study; at approximately

similar concentration, all brown seaweeds had higher activity with moderate inhibition intensity against the four species of bacteria tested.

The phenolic compounds of the dried seaweed extracts may be responsible for their antimicrobial properties. Other researchers have also reported that phenolic compounds from different plant sources could inhibit various foodborne pathogens (Smid and Gorris, 1999; Prashanth *et al.*, 2001; Kim *et al.*, 2005; Plaza *et al.*, 2009). Polyphenols, such as tannins and flavonoids, are important antibacterial substances, for example ellagitannin from pomegranates is the active substance responsible for its antimicrobial activity (Machado *et al.*, 2002).

Finally, this study utilised a substantially more sensitive detection method to quantify antimicrobial effects of seaweed extracts as compared to previous studies, which mainly relied on rather insensitive standard agar disc diffusion assays. The microtitre assay applied allowed the detection of antimicrobial effects of algal metabolites at and below algal tissue level concentrations which would otherwise have been undetected in the agar disc diffusion assay (Dubber and Harder, 2008).

4.3.5 Extraction efficiencies using methanol, ethanol and acetone on the antioxidant and antimicrobial activity

The dried methanolic extracts of three brown species of seaweeds were found to have good antioxidant and antimicrobial activity. Although a variety of solvents have been employed in screening algae for antioxidant and antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction from seaweeds (Zheng *et al.*, 2001). In order to potentially enhance the antioxidant and

antimicrobial content from red and green species, ethanol and acetone were also utilised as extraction solvents.

Phenolic compounds are generally more soluble in polar organic solvents than in water, therefore the effective extractants recommended are aqueous mixtures of methanol, ethanol and acetone (Waterman and Mole, 1994). With such polar solvents, phenolic compounds which are attached to sugar or protein, saponins, glycosides, organic acids, tannins, salts, and mucus can be extracted (Cho *et al.*, 2007). The extraction efficiencies of methanol, ethanol and acetone on total phenolic content and antioxidant activity of the red and green seaweed species are presented in Table 4.7. The total phenolic content of methanol extracts of *P. palmata* at a concentration of 1 mg/ml extract was 42.83 mg GAE/g (Table 4.7a) which was significantly higher than that of acetone (38 mg GAE/g) and ethanol (30 mg GAE/g extract). However, there was no significant difference in the antioxidant activity of methanol, ethanol and acetone extracts of *P. palmata*, which were all 25 µg/ml ($P < 0.05$).

Methanol was also the best solvent for extraction from *C. crispus* (Table 4.7b), showing a total phenolic content of 62.33 mg GAE/g and an EC₅₀ level for antioxidant activity at 5 µg/ml which was half that of the ethanol and acetone extracts ($P < 0.05$). *E. spirulina* (Table 4.7c) had a significantly higher total phenolic content from methanol extracts (49.75 mg GAE/g) than ethanol and acetone extracts which was 24.5 mg GAE/g extract for both ($P < 0.05$). Also there was no significant difference in the antioxidant activity with all solvents giving EC₅₀ levels at 50 µg/ml ($P < 0.05$).

As discussed, methanol extracts of red and green seaweeds had higher or the same total phenolic content and DPPH radical scavenging activity as ethanol and acetone extracts. This implies that the bioactive metabolite in algae might be readily soluble in methanol and slightly more sparingly soluble in ethanol and acetone. Extracts of red seaweeds have been reported to exhibit weak DPPH radical quenching activity when obtained using water, ethanol or methanol as solvents (Matsukawa *et al.*, 1997; Han *et al.*, 1999), while chloroform, ethyl acetate and acetone extracts from several red seaweeds have been reported to exhibit strong DPPH quenching activity (Yan *et al.*, 1998). Therefore, the particular solvent used to extract the seaweed material will have a dramatic effect on the compounds in the extract recovered. For example, the non-polar fraction from chloroform: methanol extraction of *Porphyra tenera* exhibited a significant protective effect against lipid oxidation associated with phospholipids such as, phosphatidyl choline and phosphatidyl ethanolamine (Kaneda and Ando, 1971). Whereas, Nakayama *et al.* (1999) reported that hexane, chloroform and methanol extracts of red seaweed, *Porphyra yezoensis* exhibited antioxidant activities attributed to the presence of β -carotene, chlorophyll analogues and amino compounds. Overall, methanol was the most effective solvent for extraction of antioxidant properties from seaweeds, which may be due to methanol having a higher dielectric constant than ethanol and acetone.

Table 4.7 Extraction efficiencies of methanol, ethanol and acetone on total phenolic content and antioxidant activity of *P. palmata* (4.7a), *C. crispus* (4.7b) and *E. spirulina* (4.7c)

(4.7a)

Solvent (60%)	Total phenolic content (mg GAE/g)	Antioxidant activity EC₅₀^a
Methanol	42.83±3.26 ^a	25.00±0.06 ^a
Ethanol	30.00±0.00 ^b	25.00±0.35 ^a
Acetone	38.00±2.82 ^c	25.00±1.12 ^a

(4.7b)

Solvent (60%)	Total phenolic content (mg GAE/g)	Antioxidant activity EC₅₀^a
Methanol	62.33±1.04 ^{ab}	5.00±0.04 ^a
Ethanol	61.00±2.82 ^a	10.00±0.64 ^b
Acetone	62.00±2.13 ^b	10.00±0.88 ^b

(4.7c)

Solvent (%)	Total phenolic content (mg GAE/g)	Antioxidant activity EC₅₀^a
Methanol	49.75±0.41 ^a	50.00±0.04 ^a
Ethanol	24.50±0.54 ^b	50.00±0.19 ^a
Acetone	24.50±0.07 ^b	50.00±0.72 ^a

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-c) differ significantly ($P < 0.05$).

^arefers to µg/ ml of extract required to reduce DPPH radical by 50% (EC₅₀)

The extract efficiencies of methanol, ethanol and acetone on antimicrobial activity of red and green seaweeds are outlined in Table 4.8. Methanolic extracts of *P. palmata* (Table 4.8a) had double the antimicrobial activity against *L. monocytogenes* (62.09%) than acetone and ethanol extracts ($P < 0.05$). In the case of *S. abony*, acetone extracts of *P. palmata* had the highest antimicrobial activity (23.39%), while for inhibiting *P. aeruginosa*, ethanol extracts were most effective showing 93.89% inhibition which was significantly higher than that of methanol ($P < 0.05$). There was no significant difference in the antimicrobial activity of ethanol and acetone extracts of *P. palmata* against *E. faecalis* both achieving 100% inhibition, however, methanolic extracts were significantly lower with only 2.21% inhibition.

Acetone extracts of *C. crispus* (Table 4.8b) had a significantly higher antimicrobial activity against *E. faecalis*, *P. aeruginosa* and *L. monocytogenes* than methanol extracts ($P < 0.05$). There was a much lower activity against *S. abony*; for all the extraction solvents tested, with ethanol providing a maximum of 0.8% inhibition. However, ethanol significantly increased the inhibition against *E. faecalis*, from a 66.08% increase in growth with methanol, to 100% with ethanol as solvent. This was a significant finding and implies that antimicrobial properties are substantially affected by solvent selection.

Ethanol was the most effective solvent for extraction from *E. spirulina* (Table 4.8c) against *E. faecalis* and *L. monocytogenes* (97.58 and 81.95% inhibition, respectively); and acetone was the most effective extraction solvent against *P. aeruginosa* and *S. abony* with 91.3 and 68.26% inhibition, respectively ($P < 0.05$). The use of ethanol and acetone increased the antimicrobial activity of the seaweed

extracts significantly dependant on the bacteria. *P. palmata* in particular had weak activity against *E. faecalis* and *P. aeruginosa*, however, with ethanol extraction, inhibition of bacteria increased to 100 and 93.89%, respectively. Acetone was a significantly better solvent against *E. faecalis* and *P. aeruginosa* with 89.74 and 81.74% inhibition, respectively. This was an interesting finding as with methanol extraction *C. crispus* extracts were enhancing the growth of the bacteria up to 66.08%. Methanolic extracts of *E. spirulina* were also weak but with ethanol and acetone as solvents they were increased to moderate and strong. The results imply that the bioactive secondary metabolite in the red and green seaweeds might be more soluble in ethanol and acetone and less soluble in methanol.

Previous reports on the most effective solvent for the extraction of antimicrobials have been varied; Gonzalez del Val *et al.* (2001) selected methanol as solvent for extraction of antimicrobial compounds from red, green and brown seaweeds. Shanmughapriya *et al.* (2008) found methanol:toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae. The same author also reported that ethanolic extracts had no antibacterial activity. Whereas Parekh *et al.* (1984) reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antimicrobial activity than chloroform extracts. Plaza *et al.* (2009) found significant differences in the antimicrobial activity depending on the solvent used. These authors found that extracts obtained using ethanol were more active than those obtained with hexane. Plaza *et al.* (2009) also identified several volatile compounds in ethanol extracts from brown seaweeds including *H. elongata*. The compounds which were characterized included fatty acids, alkanes, phenols and compounds such as phytol (2-hexadecen-1-ol,3,7,11,15-tetramethyl) and neophytadiene.

Antimicrobial activity has usually been attributed to long-chain unsaturated fatty acids (C16-C20), including palmitoleic, oleic, linoleic and linolenic acids, while long chain saturated fatty acids, including palmitic and stearic acids were less effective (Zheng *et al.*, 2005).

Table 4.8 Extraction efficiencies of methanol, ethanol and acetone on antimicrobial activity of *P. palmata* (4.8a), *C. crispus* (4.8b) and *E. spirulina* (4.8c) against selected food pathogenic ^(a) and food spoilage ^(b) bacteria

(4.8a)

Solvent	<i>L. monocytogenes</i>^a	<i>S. abony</i>^a	<i>E. faecalis</i>^b	<i>P. aeruginosa</i>^b
methanol	62.09±4.11 ^a	2.21±0.87 ^a	39.28±4.36 ^a	19.22±3.60 ^a
ethanol	37.78±1.70 ^b	0.05±1.99 ^b	100.00±2.77 ^b	93.89±3.87 ^b
acetone	37.94±2.93 ^b	23.39±4.13 ^c	100.00±4.01 ^b	78.34±2.30 ^c

(4.8b)

Solvent	<i>L. monocytogenes</i>^a	<i>S. abony</i>^a	<i>E. faecalis</i>^b	<i>P. aeruginosa</i>^b
methanol	-3.88±1.36 ^a	-10.70±0.96 ^a	-66.08±3.89 ^a	-31.72±1.52 ^a
ethanol	50.27±2.26 ^b	0.80±2.87 ^b	100.00±0.38 ^b	61.51±5.34 ^b
acetone	56.13±2.85 ^c	-4.70±2.02 ^c	89.74±2.68 ^c	81.74±4.96 ^c

(4.8c)

Solvent	<i>L. monocytogenes</i>^a	<i>S. abony</i>^a	<i>E. faecalis</i>^b	<i>P. aeruginosa</i>^b
methanol	44.72±1.77 ^a	21.21±3.69 ^a	24.13±1.18 ^a	12.45±3.72 ^a
ethanol	81.95±4.30 ^b	63.36±3.19 ^b	97.58±1.45 ^b	65.63±2.09 ^b
acetone	100.00±2.53 ^c	98.26±3.53 ^c	94.36±1.79 ^c	91.30±2.65 ^c

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-c) differ significantly ($P < 0.05$).

These results indicate that the extraction solvent has a definite effect on the isolation of bioactives. The antimicrobial inhibitory activity was only observed in the extracts obtained with a particular solvent (ethanol and acetone) but not as high in those obtained with methanol. This suggests that a particular solvent is required to extract some antimicrobial substances within the algal plant; however ethanol and acetone had no significant effect on increasing the total phenolic content and antioxidant properties of the dried seaweed extracts.

4.4 Conclusion

The results of the present chapter indicated that Irish seaweeds; *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina*, successfully displayed antioxidant and antimicrobial activities and contained phenolics, condensed tannins and flavonoids. *H. elongata* had the highest antimicrobial and antioxidant content. The extraction of antimicrobials from the different species of seaweeds was solvent dependent. Methanol is a good solvent for extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species. The antioxidant content of the seaweeds did not vary significantly with different polar solvents. This is a promising finding, as there may be a potential to utilise such extracts in food products to act as antioxidants which could enhance food quality, and also as antimicrobial agents, which could potentially increase the shelf life and safety of a wide range of food products.

CHAPTER 5

EFFECT OF DIFFERENT PROCESSING CONDITIONS ON ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *H.* *ELONGATA*

*Comparison of the effects of thermal processing such as drying, boiling, steaming,
and microwaving on the antioxidant and antimicrobial activity of H. elongata*

*The results from this chapter were published as a peer-reviewed article in the
Journal of Food Processing and Preservation*

Summary

This chapter was based on a preliminary screening aimed at investigating the effects of various thermal processing methods on the phytochemical content and activity of *H. elongata*. This seaweed was concentrated on as it showed the highest levels of antioxidants and antimicrobials in comparison to the other seaweeds species studied as illustrated in the previous chapter. Common food processing methods such as drying, boiling, steaming, microwaving and combinations of drying as a pre-treatment before hydrothermal treatments (boiling and steaming) were carried out. Each of the processing methods were carried out until the seaweed was of an edible texture (up to 50 mins). The total phenolic content (TPC) of fresh *H. elongata* was 175.27 mg GAE/100 g fresh weight (fw) while boiling significantly reduced the TPC to 25.4 mg GAE/100 g fw ($P < 0.05$). A drying pre-treatment (12 and 24 h at 25 °C) before boiling reduced the time taken to reach an edible texture, therefore leading to less leaching of antioxidants. In terms of extract, drying of *H. elongata* followed by boiling had the most significant effect on the phytochemicals as TPC increased by 174%. Of all processing treatments, extracts from boiled seaweeds had the most effective DPPH scavenging activity (EC_{50} of 12.5 µg/ml). As a comparison, *H. elongata* subjected to the same treatments was studied in terms of antimicrobial activity against *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Overall, extracts from fresh *H. elongata* exhibited the highest inhibition against the studied bacteria with 100, 87.03, 100 and 96.39% inhibition against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa*, respectively.

5.1 Introduction

Interest in new sources of natural antioxidants and antimicrobials have increased in recent years in order to reduce the use of synthetic forms such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT). Natural antioxidants from plant origin can react rapidly with free radicals and retard or alleviate the extent of oxidative deterioration (Akoh and Min, 1997). Furthermore, antioxidants from natural sources can also increase the shelf life of foods. Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular diseases (Amic *et al.*, 2003). Therefore, the consumption of foods with high levels of these phytochemicals or addition of such extracts could protect the body as well as the foods against these events (Chandini *et al.*, 2008).

Marine algae have been consumed in Asia since ancient times, but to a much lesser extent in the rest of the world. Many plant-based foods can be eaten raw or after processing. The processing can be performed in various ways but, for vegetables, most common are steaming, boiling and microwaving. These processing methods would bring about a number of changes in physical characteristics and chemical composition of the vegetables (Zhang and Hamazu, 2004). Reports on the effects of processing on the antioxidant compounds in vegetables have been inconclusive. There are reports demonstrating an enhancement or no change in antioxidant activity of vegetables (Gahler *et al.*, 2003; Turkmen *et al.*, 2005) while others have indicated a deterioration of activity after thermal treatment (Ismail *et al.*, 2004; Zhang and Hamazu, 2004).

The presence and diversity of phytochemicals in vegetables are important factors for human health. The phytochemical contents in untreated vegetables have been the most studied. Since a large part of ingested vegetables are generally thermally processed prior to consumption, it is also important to investigate how the processing and in particular heat affects the levels of these compounds (Volden *et al.*, 2009). Processing of vegetables for consumption exposes the phytochemicals present to detrimental factors that may lead to alterations in concentrations and health related quality. For example wet-thermal treatment causes denaturation of enzymes that can catalyse breakdown of nutrients and phytochemicals. On the other hand, processing by heat can result in reduction of constituents by leaching or due to thermal destruction (Rungapamestry *et al.*, 2007).

Turkmen *et al.* (2005) revealed that different processing methods (boiling, steaming and microwaving) caused losses of phenolics from squash, peas and leek. However, under similar conditions, an increase in the phenolic content of vegetables such as green beans, peppers and broccoli was reported (Turkmen *et al.*, 2005). Watchtel-Galor *et al.* (2008) found that steaming and microwaving led to losses in the total phenolic content of broccoli, choy-sum and cabbage, although steaming showed significantly less loss than microwaved samples. Volden *et al.* (2009) also reported loss of phytochemicals in steamed cauliflower (19%).

The traditional process to preserve seaweeds is by sun drying (Lim and Murtijaya, 2007) as several seaweeds are perishable in their fresh state and could deteriorate within a few days after harvest. Drying is one of the most common food processing methods that can be used to extend the shelf-life and to achieve the desired

characteristics of a food product. Reducing the water activity (a_w) of food via this process can minimize deterioration from chemical reactions and microbial activity (Chiewchan *et al.*, 2010). Dried seaweeds are rehydrated by various methods such as boiling before consumption, therefore in the present chapter drying was considered as a pre-treatment before any further hydrothermal processing.

Food poisoning is a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety regulators and regulatory agencies are continuously concerned with the high and growing number of illness or outbreaks caused by some pathogenic and spoilage microorganisms in foods. Consumers are now demanding foods which are fresh, natural and minimally processed. Along with this, consumers are also concerned about the safety of foods containing synthetic preservatives. This has put pressure on the food industry and has fuelled research into the discovery of alternative natural antimicrobials (Shan *et al.*, 2007).

Being rich in phytochemicals responsible for antioxidant and antimicrobial activity, there have been many studies conducted on seaweeds to quantify these compounds (Duan *et al.*, 2006; Chandini *et al.*, 2008 and Cox *et al.*, 2010), however little information is available on the effect of hydrothermal treatment on these phytochemicals in seaweeds.

The main aims of this chapter were as follows:

1. To carry out a preliminary screening to investigate the effect of different heat processing methods on the antioxidant constituents present in terms of whole *H. elongata* and *H. elongata* extracts.
2. To assess the antimicrobial properties of *H. elongata* extracts which had been subjected to different processing treatments against common food pathogenic and food spoilage bacteria.

5.2 Materials and methods

5.2.1 Seaweed material

Raw material is described in section 3.1. The *H. elongata* samples studied in this chapter was collected in September and November 2009 and stored at 4 °C until analysis.

5.2.2 Preparation of samples

H. elongata was washed thoroughly with tap water, dried with absorbent paper and then cut into 3 cm long pieces before processing. The effect of processing on *H. elongata* in terms of antioxidant and antimicrobial activity was evaluated by drying, boiling, steaming, microwaving, and combinations of drying as a pre-treatment before boiling and steaming. Hydrothermal processing times were decided upon as described in section 5.2.3.

5.2.3 Determination of hydrothermal processing time and texture evaluation

Hydrothermal processing time of seaweed was selected from preliminary experiments and the resulting texture was determined by the tactile method. To overcome its subjectivity, a combination of tactile and instrumental textural methods were used in order to decide the processing time of seaweed. Edible texture was determined by a sensory panel consisting of 6 judges. At 5 min intervals, boiled seaweed samples were removed from a waterbath to undergo tactile and instrumental texture analysis. Shear tests were performed as described in section 3.9.

5.2.4 Processing treatments

5.2.4.1 Drying pre-treatment

Dehydration was carried out as described in section 3.12.1 with the following modifications: samples were dried at 25 °C for 12 and 24 hours.

5.2.4.2 Boiling

The seaweed samples (dried or fresh) were boiled by immersion in 2 L of distilled water kept at the specified boiling temperatures (80 and 100 °C) using a water bath (Lauda, Aqualine AL5, Mason Technology, Ireland) until an edible texture was achieved (30 – 32 N/mm) as described in section 5.2.3. After boiling, the processed seaweeds were drained using a wire mesh strainer and placed on ice to cool before the extraction procedure.

5.2.4.3 Steaming

Steaming was carried out as described in section 3.12.3. Steaming time was selected according to preliminary experiments when an edible texture was reached (30 – 32 N/mm) as described in section 5.2.3. After the steaming process, the seaweeds were drained and placed on ice to cool before the extraction procedure.

5.2.4.4 Microwaving

Microwaving was carried out as described in section 3.12.4. Processing time was selected according to preliminary experiments. After microwaving, the seaweeds were placed on ice to cool before the extraction procedure.

5.2.5 Extraction of phytochemicals

Extraction procedure is described in section 3.2.

5.2.6 Total phenolic content

The total phenolic concentration was measured as described in section 3.4.

5.2.7 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as described in section 3.7.

5.2.8 Total flavonoid content

Total flavonoid content was determined as described in section 3.5. Results were expressed as mg quercetin equivalents (QE)/100 gram fresh weight (mg QE/100 g fw) for whole seaweeds and as mg QE/g for extracts.

5.2.9 Total condensed tannin content

Total condensed tannin content was determined as described in section 3.6. Results were expressed as mg catechin equivalents (CE)/100 gram fresh weight (mg CE/100 g fw) for whole seaweeds and as mg CE/g for extracts.

5.2.10 Antimicrobial activity

5.2.10.1 Microbial culture

Microbial cultures were prepared as described in section 3.8.1.

5.2.10.2 Antimicrobial activity assay

The antimicrobial activity assays were carried out as described in section 3.8.2.

5.2.11 Statistical analysis

All experiments were performed in triplicate and replicated twice. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA) as described in section 3.23.

5.3 Results and Discussion

5.3.1 Effect of processing on total phenolic content

Increased intake of vegetables is generally associated with a reduced risk of cancer and cardiovascular disease (Kris-Etherton *et al.*, 2002). Processing and preparation of vegetables, especially thermal treatment, which are applied prior to consumption may affect the phytochemicals. Heat applications such as boiling, steaming or microwaving are common practices in the processing of food products in order to render them palatable and microbiologically safe. Since seaweed would need to undergo some heat treatment prior to usage, it was relevant to assess the effects of heat treatment on the stability of seaweed antioxidant properties.

In the present chapter, processing of *H. elongata* was carried out by three commonly used procedures and the antioxidant properties of the processed product were evaluated and compared with fresh *H. elongata* samples. The processing methods chosen (drying, boiling, steaming and microwaving) are commonly applied to food products in order to make them edible. It is a well known fact that processing time as well as cooked texture, appearance and flavour are important processing quality characteristics (Xu and Chang, 2008). Firmness or softness is one of the most important criteria in determining the acceptability of foods. *H. elongata* is quite a tough seaweed and requires long processing times to make it edible. Because sensory evaluation is based on human senses which detect myriad characteristics of material properties simultaneously, it is difficult to find a good correlation between orally perceived texture and instrumentally measured texture (Nishinari, 2004). Therefore, in the present chapter, the hydrothermal processing time of fresh *H. elongata* boiled at 100 °C was calculated using a tactile and instrumental texture measurement. This

was based on the length of time it took the seaweed to become edible. Samples were taken every 5 minutes and edibility was judged based on the hardness and chewiness of the samples until an acceptable edible texture was achieved. From these methods, it was found that softening of fresh *H. elongata* from 45 N/mm to 30 – 32 N/mm was an acceptable edible texture as can be seen in Table 5.1.

Table 5.1 Instrumental and sensory texture evaluation to determine edible texture level of processed *H. elongata*

Processing time (mins)	Instrumental texture (N/mm)	Sensory panel judgements*
0	45.33±1.30	Too tough and chewy
5	40.47±2.31	Too tough and chewy
10	37.38±1.93	Too tough and chewy
15	35.89±0.98	Too tough and chewy
20	34.62±1.61	Too tough and chewy
25	33.98±0.79	Too tough and chewy
30	32.40±0.15	Edible
35	32.11±0.74	Edible
40	30.42±1.05	Edible
45	30.22±0.95	Edible
50	30.09±1.02	Edible

*Sensory panel judgements were based on the hardness and ease of chew of the seaweed.
Each value is presented as mean ± SD (n = 6).
Seaweeds were boiled at 100 °C

The total phenolic content (TPC) of processed *H. elongata* can be seen in Fig. 5.1. Fresh *H. elongata* had a TPC of 175.27 mg GAE/100 g fw. In order to achieve an edible texture, it was necessary that *H. elongata* was boiled for 40 and 35 min at 80 °C and 100 °C, respectively. This resulted in almost 82.31 and 85.5% loss in TPC, respectively, as compared to fresh seaweed.

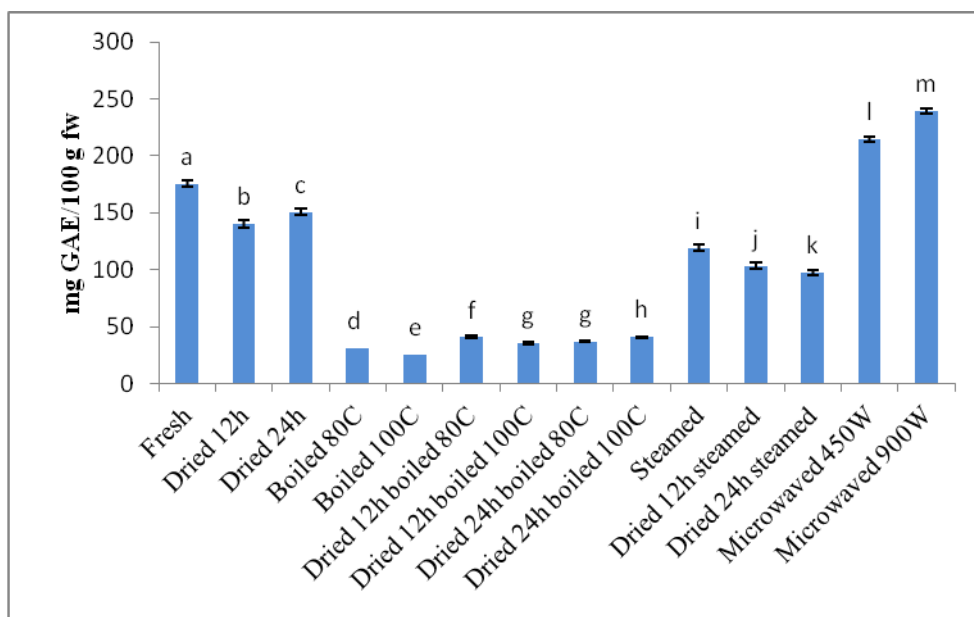


Fig. 5.1 Total phenolic content of processed *H. elongata* (mg gallic acid equivalents/100 g fw) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-m) differ significantly ($P < 0.05$)

A similar pattern was observed for steaming, although it was not as detrimental as boiling, where a loss of 32.06% in TPC was observed after 45 min of steaming. This could be due to leaching of nutrients into boiling water which was also observed by a reduction in the amount of extract obtained. However, the texture of the steamed seaweed did not reach a tender texture even after 50 min treatment. Fresh *H. elongata* had a 3.14% yield of extract whereas steaming or boiling resulted in a significant ($P < 0.05$) reduction in the yield of extract to as low as 0.26% (Table 5.2).

Table 5.2 Yield of total extract (as % w/w of seaweed on fresh weight basis), DPPH radical scavenging activity (%) of processed *H. elongata* extracts (concentration 100 µg/ml) and EC₅₀ (µg/ml)* of each extract

Processing treatment	Total methanol extract (%)	DPPH radical scavenging activity (%)	EC ₅₀ (µg/ml)
Fresh	3.14±0.19 ^a	75.50±2.30 ^a	25.00±1.81 ^a
Dried 12h	3.25±0.20 ^a	74.69±2.31 ^b	50.00±3.31 ^b
Dried 24h	3.48±0.29 ^{ab}	67.87±1.05 ^c	50.00±2.55 ^b
Boiled 80 °C 40 min	0.27±0.09 ^c	92.96±1.55 ^d	12.50±1.12 ^c
Boiled 100 °C 35 min	0.26±0.06 ^c	93.54±1.24 ^e	12.50±2.00 ^c
Dried 12h and boiled 80 °C 30 min	0.28±0.05 ^c	95.33±0.80 ^f	12.50±1.00 ^c
Dried 12h and boiled 100 °C 25 min	0.28±0.06 ^c	100.00±0.00 ^g	12.50±1.02 ^c
Dried 24h and boiled 80 °C 30 min	0.24±0.02 ^c	88.50±1.24 ^h	12.50±1.21 ^c
Dried 24h and boiled 100 °C 20 min	0.27±0.02 ^c	89.04±1.85 ⁱ	12.50±1.00 ^c
Steamed 45 min	3.37±0.21 ^a	52.51±0.56 ^j	100.00±0.98 ^d
Dried 12h and steamed 50 min	3.31±0.19 ^a	53.79±1.88 ^k	100.00±1.99 ^d
Dried 24h and steamed 50 min	3.30±0.20 ^a	53.50±0.97 ^l	100.00±0.96 ^d
Microwaved 450w - 30 s	3.75±0.55 ^b	76.29±1.57 ^m	25.00±1.52 ^a
Microwaved 900w - 20 s	4.34±0.56 ^d	75.35±2.00 ⁿ	25.00±1.88 ^a

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-n) differ significantly ($P < 0.05$).

*EC₅₀ value is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50%.

Studies have shown that phenolic compounds are sensitive to heat, whereby boiling of vegetables for few minutes could cause a significant loss of phenolic content which can leach into boiling water (Amin *et al.*, 2006). Xu and Chang (2008) reported a 40 - 50% loss in the TPC of legumes due to leaching of phenolics in boiling water whereas Oboh (2005) found up to 200% increase in the phenolic content of boiled tropical green leafy vegetables. Reduction in TPC was also found in other vegetables such as broccoli, kale and spinach (Zhang and Hamauzu, 2004;

Ismail *et al.*, 2004). These authors stated the probable reason was due to dissolution of polyphenols into water which could be the case with *H. elongata* as it requires long hydrothermal processing times to become edible. Although microwaving has been reported to be the most deleterious with respect to the antioxidant properties of vegetables (Sultana *et al.*, 2008), the results in the present study were encouraging as microwaving increased the TPC by 22.49% (450 W) and 36.58% (900 W), as compared to fresh.

In order to reduce the processing time and eventual loss of phytochemicals, drying was used as a pre-treatment. The process of drying in itself was not detrimental as a drying period of 12 h and 24 h retained 80.1 and 85.96% of the original phenolic content as compared to fresh seaweed. Possible losses could be attributed to stressing the plant during the drying process due to loss of water through the cell walls. Moreover, drying for 12 and 24 h prior to boiling not only reduced the processing time but also the loss in TPC. Drying for 24 h prior to boiling at 100 °C decreased processing time by 15 mins and reduced the loss of total phenols by 8.83%, as compared to boiling at 100 °C without a drying pre-treatment. Drying as a pre-treatment was not effective before steaming as 44.19% reduction in TPC was seen when seaweed was dried for 24 h followed by steaming ($P < 0.05$). Microwaving was not carried out on dried seaweeds as water was not used, therefore rehydration would not take place. Although heat processing seriously degraded the quality of seaweed, an interesting observation was an increase in the potency of the extract in samples which were boiled (Fig. 5.2).

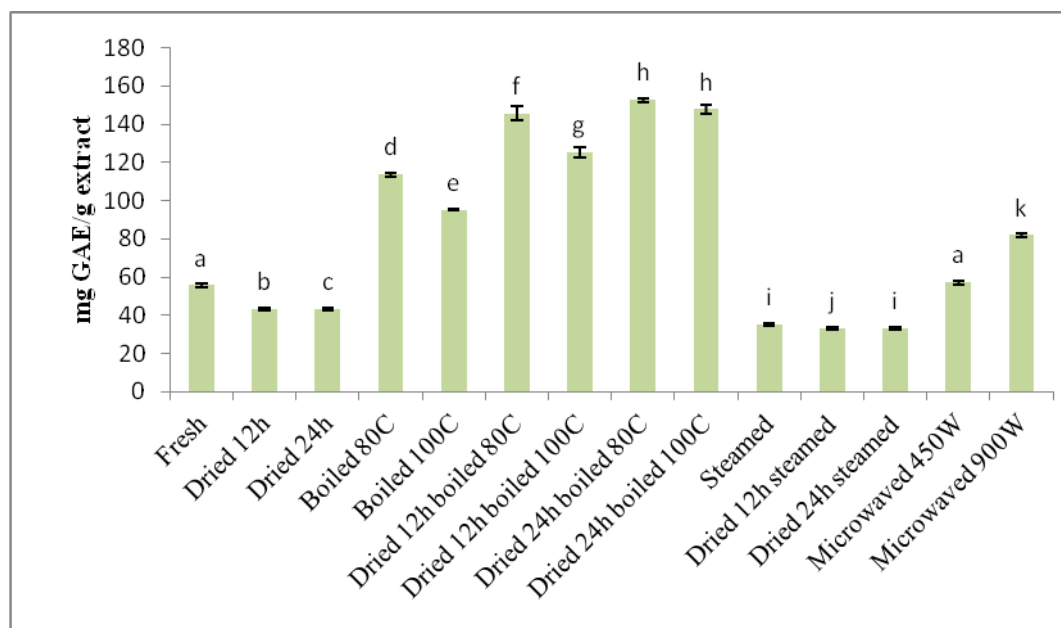


Fig. 5.2 Total phenolic content of processed *H. elongata* extract (mg gallic acid equivalents/g extract) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-k) differ significantly ($P < 0.05$).

When the activity of the heat processed extract was compared in terms of per gram of dried extract, boiling at 80 and 100 °C resulted in an increase of 104.03% and 71% TPC per g of dried extract, respectively, as compared to fresh ($P < 0.05$). Fresh *H. elongata* contained 55.75 mg GAE/g of extract while seaweed dried for 12 and 24 h led to significant reductions in TPC up to 22.42% as compared to fresh ($P < 0.05$). When boiling was applied after a drying pre-treatment for 12 h, a 161.43 and 125.11% increase per g of extract was observed at 80 and 100 °C, as compared to fresh samples ($P < 0.05$).

Samples pre-treated by drying for 24 h followed by boiling at 100 °C for 20 min increased the TPC by 165.32% as compared to fresh. Drying of *H. elongata* for 24 h

followed by boiling at 80 °C for 30 mins had the most significant effect ($P < 0.05$) on the TPC of all treatments resulting in 173.99% increase in TPC. Steaming for 45 mins had a 36.62% decrease in TPC per g of extract while a drying pre-treatment before steaming for 50 mins had a 40% decrease. Microwaving at 450 W caused no significant increase in the TPC per g of extract as compared to fresh ($P < 0.05$), while microwaving at 900 W increased the TPC to 82 mg GAE/g of extract (47.08% increase). As the TPC of *H. elongata* per g of extract was increased due to hydrothermal processing (boiling), a lower concentration of the extract would be required to have a potential effect on preventing oxidation in food products which is a promising finding. This could be due to the composition of the extracts after boiling. There are significant losses of potential bioactives and subsequent reduction in extract yield during boiling due to leaching. It is therefore possible that stronger more potent compounds remain in the extracts of such boiled seaweeds and therefore lead to an increase in the activity compared to steamed and microwaved samples which do not come in contact with water.

5.3.2 Effect of processing on DPPH radical scavenging activity

The results of the DPPH free radical scavenging ability of the seaweed processed under different heat processing conditions are shown in Table 5.2. The results indicated that free radical scavenging ability of the processed seaweeds ranged from 52.51 to 100% (concentration 100 µg/ml extract) with extracts from seaweed dried for 12 h followed by boiling at 100 °C being most effective. Significant differences ($P < 0.05$) in DPPH values were found for all processing treatments. At 100 µg/ml extract concentration, drying led to slight decrease in DPPH radical scavenging activity from 75.5% to 74.69% (12 h) and 67.87% (24 h) while boiling led to a

significant increase ($P < 0.05$). Boiling at 100 °C increased DPPH scavenging by 23.89%, from 75.5 to 93.54%. Drying of *H. elongata* for 12 h followed by boiling at 100 °C had the most significant increase in antioxidant activity as 100% inhibition of the DPPH radical was achieved with 100 µl/ml of extract. Steaming significantly reduced the DPPH radical scavenging activity ($P < 0.05$) to 52.51%. Extracts from *H. elongata* given drying pre-treatments followed by steaming had 53.79% (12 h) and 53.5% (24 h) scavenging of the DPPH radical. Seaweed microwaved at 450 W had 76.29% activity against DPPH radical while microwaving at 900 W had 75.35% activity.

DPPH results are often interpreted as the “efficient concentration” or EC_{50} value, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (Molyneux, 2004). The EC_{50} values of DPPH radical scavenging activity from dried methanolic extracts of seaweeds are also presented in Table 5.2. Processing of *H. elongata* resulted in significantly different EC_{50} values ($P < 0.05$), depending upon treatment. The EC_{50} levels ranged from 12.5 to 100 µg/ml of extract with all treatments in which boiling was found to have the most effective EC_{50} values (12.5 µg/ml of extract). Extracts from fresh seaweed had an EC_{50} of 25 µg/ml. Drying of seaweed led to a significant ($P < 0.05$) reduction in the DPPH radical scavenging activity of the extract to 50 µg/ml (12 and 24 h). Steaming had the most detrimental effect on the DPPH radical scavenging activity of the extract as 100 µg/ml was required to reduce the DPPH radical by 50% and activity at 100 µg/ml concentration was almost half that of the most effective processed seaweed (53.5 and 100%, respectively). There was no significant difference between microwaved seaweed extracts compared to fresh ($P > 0.05$) as all had an EC_{50} value of 25 µg/ml.

For similar reasons to the increase in the potency of the TPC of extracts, the DPPH activity of extracts from boiled seaweeds may have increased due to leaching of less potent constituents into boiling water whereas the steamed and microwaved samples were not in direct contact with water for such leaching to occur.

5.3.3 Effect of processing on total flavonoid content

The bioavailability of phytochemicals is influenced by the matrix and microstructure of the food they occur in, the storage conditions (light, oxygen, and temperature regime) and thermal processing they are subjected to. As a consequence, knowledge of the content and stability of phytochemicals in foods after heat processing is essential to evaluate the nutritional value of foods rich in these phytochemicals, such as seaweed (Parada and Aguilera, 2007). The total flavonoid content (TFC) of processed whole *H. elongata* is presented in Fig. 5.3.

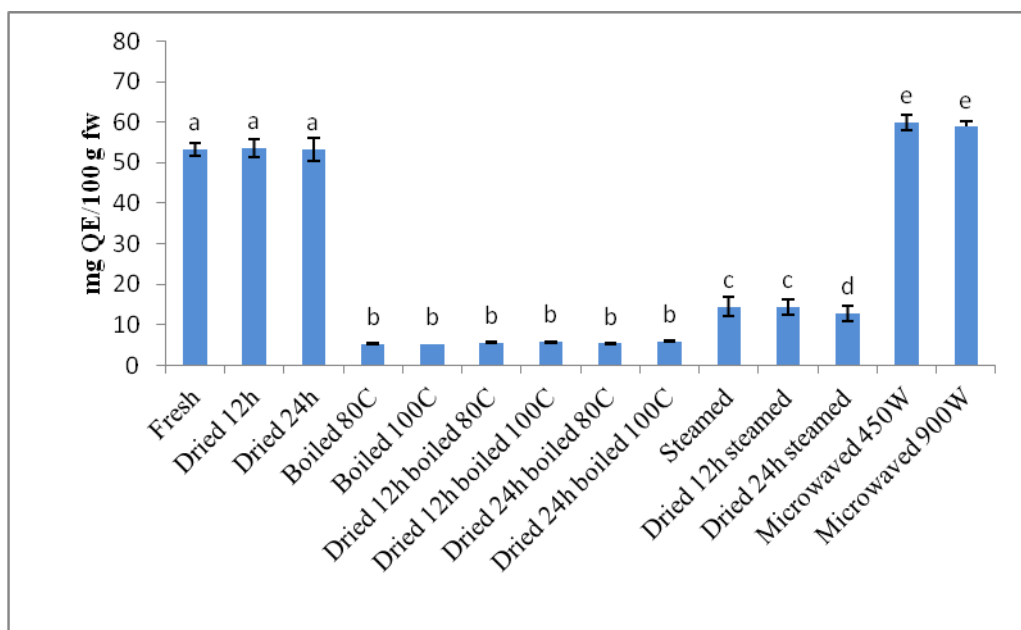


Fig. 5.3 Total flavanoid content of processed *H. elongata* (mg quercetin equivalents/100 g fw) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-e) differ significantly ($P < 0.05$).

The TFC of fresh *H. elongata* was 53.18 mg QE/100 g fw. Drying for 12 and 24 h had no significant effect on the TFC as there was only a slight increase of 0.72 and 0.25%, respectively ($P > 0.05$). All treatments which included boiling significantly reduced the TFC, within a range of 88.86 to 90.18%. This highest reduction was seen in fresh seaweed boiled at 100 °C which led to a 90.18% reduction in TFC ($P < 0.05$). Release of flavonoids and increased chemical extraction of these compounds could be induced by the effect of boiling (Olivera *et al.*, 2008). This release of flavonoids coupled with contact and leaching into water could have resulted in high reduction in TFC for boiled samples. The results of the present study are similar to Olivera *et al.* (2008) who found that boiling decreased TFC in brussels sprouts. Steaming also led to a significant reduction in TFC compared to fresh but this was

significantly lower as compared to boiled seaweeds ($P < 0.05$). Steaming retained 17.4% more TFC than boiled samples. This is most likely due to the fact that steamed seaweeds were not in direct contact with water which resulted in considerably less leaching of flavonoids. These results are in line with Francisco *et al.* (2010) and Rodrigues *et al.* (2009) who also reported significant losses of flavonoids up to 67% in processed conventional vegetables. Microwaving at 450 W had a 12.69% increase in TFC while microwaving at 900 W raised the TFC by 10.65% ($P < 0.05$).

Total flavonoid content of processed *H. elongata* extracts are presented in Fig. 5.4. Extracts from fresh *H. elongata* contained 42.29 mg QE/g of extract. All treatments significantly changed the TFC content as compared to fresh ($P < 0.05$). Simple drying for 12 and 24 h significantly reduced ($P < 0.05$) the TFC in the range of 2.45% to 9.35%. Boiling at 80 and 100 °C resulted in an increase up to 15.76%, however, a combination of drying pre-treatment followed by boiling had the most significant effect on the TFC of *H. elongata*. Drying for 12 h followed by boiling at 80 and 100 °C resulted in 18.72 and 21.67% increase in TFC, respectively ($P < 0.05$). Drying for 24 h followed by boiling at 100 °C for 20 mins had a 26.6% increase in TFC. The most significant increase of 32.02% was seen in samples dried for 24 h followed by boiling at 80 °C for 30 mins. Steaming alone and in combination with 12 and 24 h drying pre-treatments resulted in 14, 11.43 and 11.98% increase in TFC, respectively. The increase in the case of microwaved samples ranged from 8.72% to 14.29%.

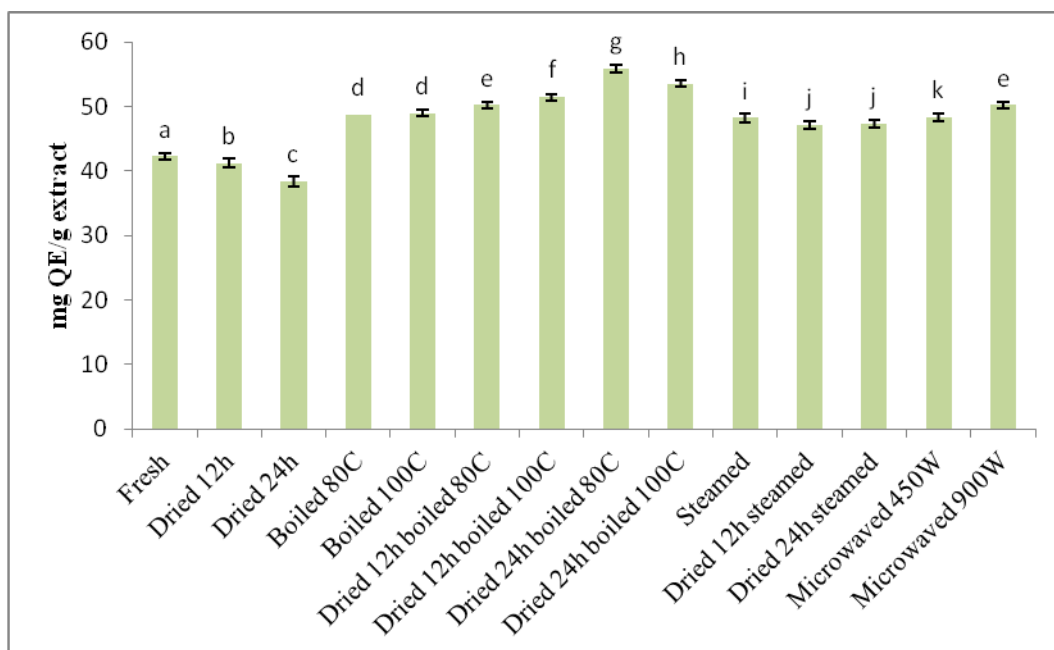


Fig. 5.4 Total flavanoid content of processed *H. elongata* extract (mg quercetin equivalents/g extract) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-k) differ significantly ($P < 0.05$).

5.3.4 Effect of processing on total condensed tannin content

Phlorotannins are a group of phenolic compounds which are restricted to polymers of phloroglucinol and have been identified from several brown algae. Many studies have shown that phlorotannins are the only phenolic group detected in brown algae (Jormalainen and Honkanen, 2004; Koivikko *et al.*, 2007). Total condensed tannin content (TTC) of processed *H. elongata* can be seen in Fig. 5.5.

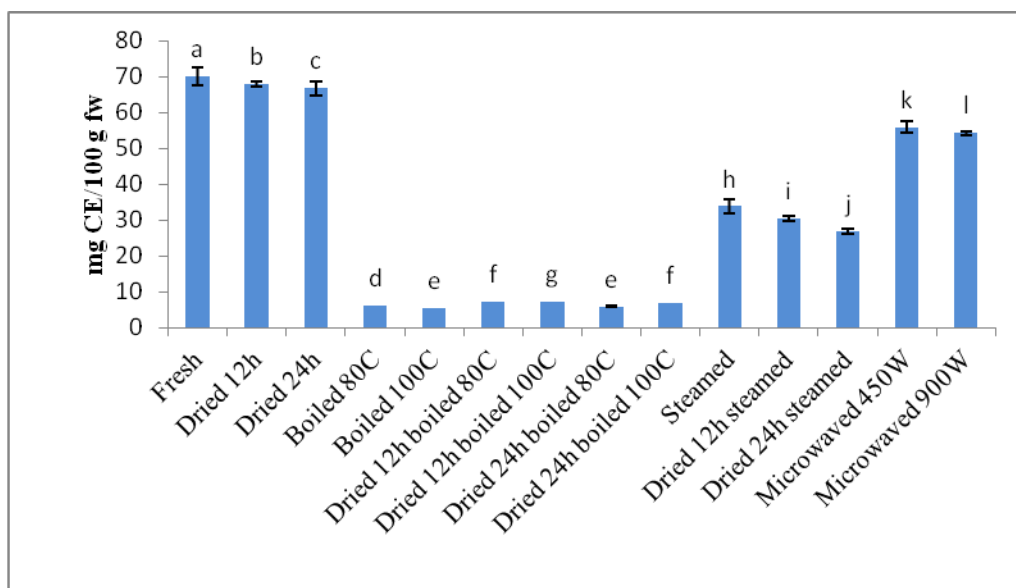


Fig. 5.5 Total condensed tannin content of processed *H. elongata* (mg catechin equivalents/100 g fw) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-l) differ significantly ($P < 0.05$).

Condensed tannins of the studied seaweeds ranged from 70.61 to 5.5 mg QE/100 g fw. Fresh *H. elongata* contained 70.05 mg CE/100 g fw while drying for 12 and 24 h reduced the TTC by 2.92 and 4.73%, respectively. Similarly to total flavonoid contents, TTC was significantly reduced upon boiling ($P < 0.05$). Boiling at 80 °C for 40 mins significantly reduced the TTC from 70.05 to 6.22 mg CE/100 g fw (91.11% reduction). The most significant reduction of 92.13% in TTC was seen in *H. elongata* boiled at 100 °C for 35 mins ($P < 0.05$). Similar to TFC, steaming had a lower reduction of TTC as compared to boiling. Steaming retained 40.5% more TTC than boiled samples as compared to fresh ($P < 0.05$). Microwaving at 450 and 900 W had a 20.27 and 22.54% reduction in TTC, respectively ($P < 0.05$). The basis of the significant decrease in processed seaweeds could also be attributed to the possible break-down of tannins present in the seaweed to simple phenol (Akindahunsi and

Oboh, 1999). Khandelwal *et al.* (2010) and Somsab *et al.* (2008) also found decreases in tannin levels of processed legumes and vegetables.

In contrast to TPC and TFC, a significant reduction in the total condensed tannins of processed (dried, boiled, steamed and microwaved) *H. elongata* extracts was observed as compared to fresh as can be seen in Fig. 5.6.

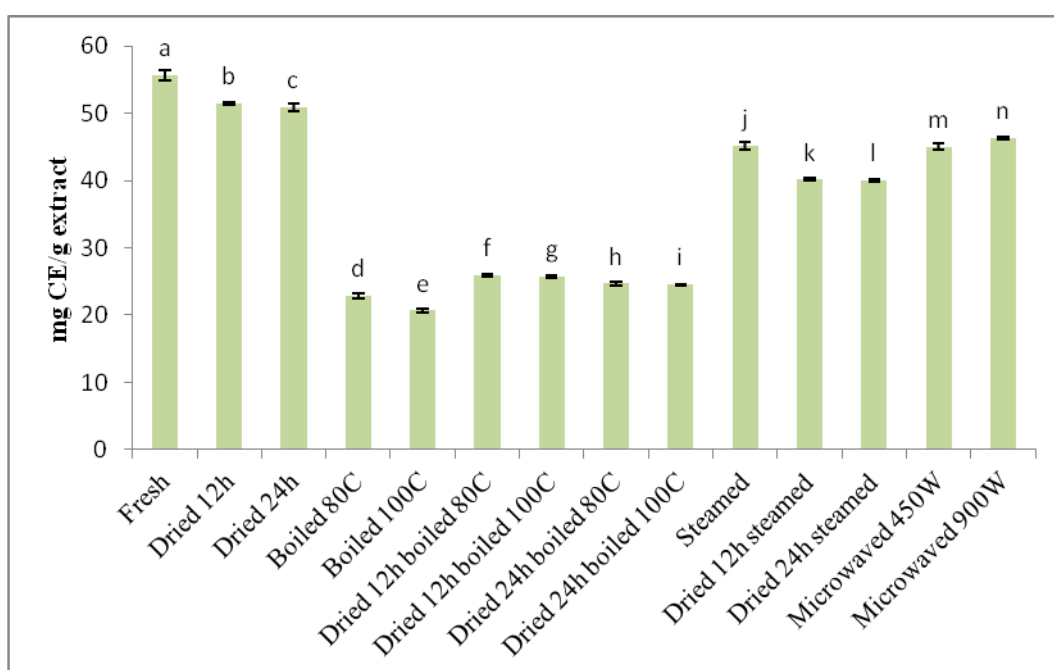


Fig. 5.6 Total condensed tannin content of processed *H. elongata* extract (mg catechin equivalents/g extract) as a function of different processing methods and conditions

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-n) differ significantly ($P < 0.05$).

Extracts from fresh *H. elongata* contained 55.7 mg CE/g, while drying for 12 and 24 h had 7.73 and 8.65% reduction in TTC, respectively. Boiling at 80 °C for 40 mins led to a 58.97% reduction in TTC while 62.89% reduction was seen in *H. elongata*

boiled at 100 °C for 35 mins. Drying pre-treatment followed by boiling also had significant losses of TTC but less than that of boiled seaweed. Drying for 12 h before boiling at 80 and 100 °C had 53.52 and 53.78% reduction respectively. Drying for 24 h followed by boiling at 80 °C for 30 min caused a loss of TTC by 55.71% while drying for 24 h in combination with boiling at 100 °C for 20 mins had a 55.91% reduction from 55.7 to 24.55 mg CE/g of extract. Steaming resulted in 18.91% reduction while in combination with a drying pre-treatment there was a 28% reduction as compared to extracts from fresh *H. elongata*. Microwaving at 450 and 900 W for 30 and 20 s had a 19.14 and 16.88% reduction in TTC, respectively ($P < 0.05$). The higher reductions in boiled samples as compared to steaming and microwaving would be due to the water solubility and leaching of the tannins into the blanching water. Many phenolics and tannins are water soluble and can be solubilised and lost (Waterman and Mole, 1994; Mueller-Harvey, 2006).

5.3.5 Antimicrobial activity of processed *H. elongata* extracts against *L. monocytogenes*

Antimicrobial activity of processed seaweed extracts was studied in order to analyse the effect of food processing on their activity. The entire yield of extract from 5 g original weight of each of the processed seaweeds were dissolved in 2.5 ml TSB and utilized in the assay. Therefore, different concentrations were obtained at each dilution level as can be seen in Table 5.3.

In the present chapter, antimicrobial activity was tested against common food spoilage (*E. faecalis* and *P. aeruginosa*) and food pathogenic (*L. monocytogenes* and *S. abony*) bacteria. These organisms were studied after discussions with the Food

Safety Authority of Ireland because they have been identified as being problematic in the Irish food industry. The entire spectrum of inhibitory effects is reported as outlined by Dubber and Harder (2008) as can be seen in Table 3.1 in section 3.8.2.

Table 5.3 Concentration of *H. elongata* extracts (mg/ml) from different heat processed seaweeds (5 g original seaweed) for each dilution tested

Processing treatment	Dilution 1 (mg/ml)	Dilution 2 (mg/ml)	Dilution 3 (mg/ml)	Dilution 4 (mg/ml)
Fresh	31.44	15.72	7.86	3.93
Dried 12h	32.47	16.23	8.12	4.06
Dried 24h	34.77	17.39	8.69	4.35
Boiled 80 °C 40 min	2.73	1.36	0.68	0.34
Boiled 100 °C 35 min	2.67	1.33	0.67	0.33
Dried 12h and boiled 80 °C 30 min	2.80	1.40	0.70	0.35
Dried 12h and boiled 100 °C 25 min	2.82	1.41	0.70	0.35
Dried 24h and boiled 80 °C 30 min	2.42	1.21	0.61	0.30
Dried 24h and boiled 100 °C 20 min	2.77	1.38	0.69	0.35
Steamed 45 min	34.00	17.00	8.50	4.25
Dried 12h and steamed 50 min	31.00	15.50	7.75	3.88
Dried 24h and steamed 50 min	29.30	14.65	7.33	3.66
Microwaved 450w - 30 s	37.56	18.78	9.39	4.69
Microwaved 900w - 20 s	43.47	21.74	10.87	5.43
Each value is presented as mean \pm SD (n = 6).				

L. monocytogenes is a Gram-positive pathogenic bacterium commonly isolated from foods in many countries including Ireland (Chitlapilly-Dass *et al.*, 2010). The percentage inhibition of the processed seaweed extracts against *L. monocytogenes* are presented in Table 5.4 and the concentrations of extract for each dilution of processed *H. elongata* are outlined in Table 5.3.

Table 5.4. Percentage inhibition of methanolic extracts of *H. elongata* heat processed under different conditions against *L. monocytogenes*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	100.00±0.0 ^{aw}	100.00±0.00 ^{aw}	99.37±1.24 ^{ax}	98.09±3.81 ^{ay}
Dried 12h	100.00±0.0 ^{aw}	89.88±1.70 ^{bx}	43.89±1.43 ^{by}	31.68±2.08 ^{bz}
Dried 24h	100.00±0.0 ^{aw}	92.99±8.16 ^{cx}	29.84±2.19 ^{cy}	26.98±6.72 ^{cz}
Boiled 80 °C 40 min	27.05±0.60 ^{bw}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}
Boiled 100 °C 35 min	46.45±3.42 ^{cw}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}
Dried 12h and boiled 80 °C 30 min	24.95±3.51 ^{dw}	10.00±1.71 ^{ex}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 12h and boiled 100 °C 25 min	21.02±2.54 ^{ew}	14.00±0.25 ^{fx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 80 °C 30 min	10.48±3.23 ^{fw}	9.81±1.15 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 100 °C 20 min	23.20±2.54 ^{gw}	15.89±1.68 ^{hx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Steamed 45 min	96.34±3.64 ^{hw}	96.24±1.56 ^{ix}	81.54±5.56 ^{ey}	77.88±5.54 ^{ez}
Dried 12h and steamed 50 min	43.25±3.23 ^{iw}	35.14±0.25 ^{ix}	29.57±1.77 ^{fy}	20.45±0.78 ^{fz}
Dried 24h and steamed 50 min	43.85±0.23 ^{jw}	34.15±1.12 ^{kx}	28.89±1.89 ^{gy}	19.55±0.98 ^{gz}
Microwaved 450w - 30 s	97.24±0.05 ^{kw}	82.90±5.90 ^{lx}	48.73±1.57 ^{hy}	29.41±5.45 ^{hz}
Microwaved 900w - 20 s	97.26±1.25 ^{ew}	81.45±3.07 ^{mx}	74.20±1.84 ^{iy}	72.86±3.10 ^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-m) differ significantly ($P < 0.05$).

Means within each row with different letters (w-z) differ significantly ($P < 0.05$).

At highest extract concentrations, extracts from fresh seaweed and those dried for 12 and 24 h (31.44, 32.47 and 34.77 mg/ml, respectively) had 100% inhibition against *L. monocytogenes*. Any processing treatment which included boiling of *H. elongata* had weak activity against *L. monocytogenes* (< 25% inhibition), which could be due to leaching of phytochemicals during boiling resulting in lower concentrations. Extracts of steamed seaweed had strong activity against *L. monocytogenes* at the highest dilution tested (96.34% inhibition at 34 mg/ml). However when drying was applied as a pre-treatment before steaming there was less than half the inhibition activity against *L. monocytogenes*; 43.25% (31 mg/ml) and 43.85% (29.3 mg/ml) for 12 and 24 h dried samples, respectively. Microwaving at 450 and 900 W produced extracts with strong inhibition against *L. monocytogenes* in the first dilution (97.24 and 97.26% inhibition at 37.56 and 43.37 mg/ml extract, respectively). As the yield of microwaved *H. elongata* extract was higher than other treatments, this would suggest that the extract is in fact slightly less potent than those of fresh seaweed.

There was no significant difference ($P > 0.05$) between the first and second dilutions of fresh *H. elongata* extract tested against *L. monocytogenes* (15.72 mg/ml) which again had 100% inhibition in the second dilution. An inhibition activity of 89.88 to 92.99% was obtained by dried extracts whereas extracts from boiled seaweeds had completely lost the antimicrobial activity. Drying pre-treatment before boiling seemed to maintain weak antimicrobial activity of the extracts in the range of 9.81 to 15.89% in the second dilution as compared to 0%. Extracts from steamed seaweed at the second dilution of 17 mg/ml had strong activity against *L. monocytogenes* giving 96.24% inhibition. Seaweeds which received a drying pre-treatment (12 and 24 h) before steaming had significantly less antimicrobial activity against *L.*

monocytogenes in the second dilution with inhibition levels of 34.14 and 34.15% at 15.5 and 14.65 mg/ml, respectively ($P > 0.05$). Extracts from the second dilution of microwaved seaweed had 82.9% (450 W) and 81.45% (900 W) inhibition against *L. monocytogenes* at 18.78 and 21.74 mg/ml extract concentrations, respectively. At the third and fourth dilutions; although fresh seaweeds still had 98% activity, all processed seaweeds had significantly less inhibition ($P < 0.05$).

5.3.6 Antimicrobial activity of processed *H. elongata* extracts against *S. abony*

Salmonella is a Gram-negative food-borne pathogenic bacterium and has become one of the most important causes of acute enterocolitis throughout the world. Salmonellosis is caused by any of over 2300 serovars of *Salmonella* (Wong *et al.*, 2000; Lee *et al.*, 2001). Fresh *H. elongata* had the highest activity against *S. abony* as can be seen in Table 5.5. However, overall, the processed extracts were least effective against *S. abony*, and no processing treatment achieved 100% inhibition of the bacteria. A potential cause is that *S. abony* is Gram-negative, as there are significant differences in the outer layers of Gram-negative and Gram-positive bacteria cell structure. Gram-negative bacteria possess an outer membrane and a unique periplasmic space which is not found in Gram-positive bacteria (Nikaido, 1996; Duffy and Power, 2001). Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Kalembe and Kunicka, 2003).

Table 5.5 Percentage inhibition of methanolic extracts of *H. elongata* processed under different conditions against *S. abony*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	87.03±3.91 ^{aw}	78.80±1.41 ^{ax}	65.13±4.60 ^{ay}	37.18±5.71 ^{az}
Dried 12h	77.29±1.26 ^{bw}	39.24±2.27 ^{bx}	26.83±3.75 ^{by}	11.01±2.06 ^{bz}
Dried 24h	83.52±4.23 ^{cw}	49.01±3.08 ^{cx}	27.17±4.02 ^{cy}	10.99±1.07 ^{cz}
Boiled 80 °C 40 min	10.37±3.97 ^{dw}	5.50±0.12 ^{dx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Boiled 100 °C 35 min	16.56±4.11 ^{ew}	0.00±0.00 ^{ex}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}
Dried 12h and boiled 80 °C 30 min	13.73±1.09 ^{fw}	10.34±0.89 ^{fx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 12h and boiled 100 °C 25 min	13.87±1.69 ^{gw}	6.01±1.29 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 80 °C 30 min	22.11±2.98 ^{hw}	10.61±1.68 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 100 °C 20 min	15.54±1.75 ^{iw}	11.02±4.87 ^{hx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Steamed 45 min	93.23±2.51 ^{jw}	93.04±2.51 ^{ix}	82.45±4.25 ^{ey}	77.12±4.45 ^{ez}
Dried 12h and steamed 50 min	44.21±3.78 ^{kw}	35.78±0.29 ^{jx}	29.87±2.51 ^{fy}	20.12±2.58 ^{fz}
Dried 24h and steamed 50 min	44.12±2.53 ^{kw}	36.15±1.25 ^{kx}	28.78±1.88 ^{gy}	19.70±1.80 ^{gz}
Microwaved 450w - 30 s	93.00±0.98 ^{lw}	58.65±1.29 ^{lx}	48.62±1.58 ^{hy}	30.64±1.35 ^{hz}
Microwaved 900w - 20 s	94.40±1.58 ^{mw}	64.09±2.26 ^{mx}	53.07±5.98 ^{iy}	31.28±3.78 ^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-m) differ significantly ($P < 0.05$).

Means within each row with different letters (w-z) differ significantly ($P < 0.05$).

Fresh *H. elongata* extracts had activity which ranged from 87.03 to 37.18% in the first to fourth dilutions. Seaweed which had been dried for 12 and 24 h had moderate activity against *S. abony* in the first dilution of extract but had weak activity from the second dilution onwards ($< 50\%$ inhibition). Any processing treatment which included boiling resulted in weak activity against *S. abony* with a maximum of 22.11% inhibition. Extracts from steamed *H. elongata* had strong activity in the first and second dilutions ($> 90\%$) and moderate activity thereafter. Drying of seaweed before steaming led to a significant reduction ($P < 0.05$) in antimicrobial activity as compared to steaming alone against *S. abony* (44% inhibition) in the first dilution and weak activity in subsequent dilutions. Activity of microwaved *H. elongata* extracts at 450 and 900 W ranged from strong ($\leq 94.4\%$) to weak ($< 50\%$) against *S. abony*.

5.3.7 Antimicrobial activity of processed *H. elongata* extracts against *E. faecalis*

The Gram-positive bacterium *E. faecalis* is a natural member of the human and animal gastrointestinal flora. This bacterium is an indicator of faecal contamination and has been detected in food, milk and drinking water (Rincé *et al.*, 2003). Antimicrobial activity of processed *H. elongata* extracts against *E. faecalis* are outlined in Table 5.6.

Table 5.6 Percentage inhibition of methanolic extracts of *H. elongata* processed under different conditions against *E. faecalis*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	100.00±0.00 ^{aw}	99.61±0.66 ^{ax}	87.03±1.41 ^{ay}	78.02±0.98 ^{az}
Dried 12h	100.00±0.00 ^{aw}	39.24±2.27 ^{bx}	36.30±6.50 ^{by}	33.89±5.10 ^{bz}
Dried 24h	95.40±0.72 ^{bw}	53.01±3.08 ^{cx}	42.28±0.08 ^{cy}	29.96±1.38 ^{cz}
Boiled 80 °C 40 min	7.31±1.25 ^{cw}	5.50±0.12 ^{dx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Boiled 100 °C 35 min	10.52±2.54 ^{dw}	0.00±0.00 ^{ex}	0.00±0.00 ^{dx}	0.00±0.00 ^{dx}
Dried 12h and boiled 80 °C 30 min	49.45±1.26 ^{ew}	10.34±0.89 ^{fx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 12h and boiled 100 °C 25 min	22.11±4.32 ^{fw}	6.01±1.29 ^{dx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 80 °C 30 min	16.67±2.46 ^{gw}	10.61±1.68 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 100 °C 20 min	33.72±3.92 ^{hw}	11.02±4.87 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Steamed 45 min	95.21±1.29 ^{iw}	93.54±2.51 ^{hx}	81.47±1.58 ^{ey}	76.42±2.57 ^{ez}
Dried 12h and steamed 50 min	42.32±5.29 ^{iw}	35.78±0.29 ^{ix}	28.74±2.50 ^{fy}	19.99±1.54 ^{fz}
Dried 24h and steamed 50 min	44.57±0.98 ^{kw}	36.15±1.25 ^{jx}	29.87±1.75 ^{gy}	18.78±1.24 ^{gz}
Microwaved 450w - 30 s	90.12±2.54 ^{lw}	48.62±1.29 ^{kx}	31.13±2.01 ^{hy}	23.48±2.06 ^{hz}
Microwaved 900w - 20 s	95.81±4.58 ^{mw}	53.07±2.26 ^{cx}	43.61±3.39 ^{iy}	24.42±5.14 ^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-m) differ significantly ($P < 0.05$).

Means within each row with different letters (w-z) differ significantly ($P < 0.05$).

Fresh *H. elongata* extracts had more than 99% antimicrobial activity until the second dilution and moderate activity in the third and fourth dilutions (87.03 and 78.02%, respectively). Dried seaweed extracts had very strong (100%) and strong (95.4%) activity in the first dilution for 12 and 24 h, respectively, however this level was halved in the second dilution with 39.24% (12 h) and 53.01% inhibition (24 h). Extracts from boiled seaweeds had less than 10% activity in the first two dilutions and was completely lost in lower dilutions. A drying pre-treatment before boiling retained more of the bioactivity ($P < 0.05$) of the extract, for example extracts of *H. elongata* processed by drying for 12 h followed by boiling at 80 °C for 30 mins had 51.45% inhibition. Extracts from steamed *H. elongata* had strong activity in the first and second dilutions ($\leq 95.21\%$) and moderate activity thereafter. Drying of seaweed before steaming led to a significant reduction ($P < 0.05$) in antimicrobial activity as compared to steaming alone against *E. faecalis* also ($\leq 44.57\%$ inhibition). Extracts of microwaved *H. elongata* had strong activity in the first dilution ($\leq 95.81\%$) while extracts from the second dilution onwards were significantly less effective with activity below 53.07%.

5.3.8 Antimicrobial activity of processed *H. elongata* extracts against *P. aeruginosa*

P. aeruginosa is a ubiquitous Gram-negative food spoilage bacterium with great adaptability and metabolic versatility. *P. aeruginosa* can attach onto a variety of surfaces and in a variety of niches including the food processing environments by forming biofilms, which are more resistant to environmental stresses, host-mediated responses, sanitizing agents, and antimicrobial agents (Bremer *et al.*, 2001). Antimicrobial activity of processed *H. elongata* extracts against *P. aeruginosa* are

presented in Table 5.7. Similar to Gram-negative *S. abony*, there was also no processed seaweed extract which gave 100% inhibition against *P. aeruginosa*. Antimicrobial activity of extracts from fresh *H. elongata* were strong in the first dilution with 96.39% inhibition and moderate in the subsequent dilutions. Seaweed which had been dried for 12 and 24 h achieved a maximum activity of 94.05%. Boiling led to a significant reduction in antimicrobial activity of extracts against *P. aeruginosa*. Activity of steamed *H. elongata* extracts ranged from 95.32 to 77.54% in the first to fourth dilutions. Extracts from seaweed given a drying pre-treatment followed by steaming had less than half the level of activity as compared to steaming alone, possibly due to there being a reduction at both the drying and steaming stages.

Table 5.7 Percentage inhibition of methanolic extracts of *H. elongata* processed under different conditions against *P. aeruginosa*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	96.39±2.55 ^{aw}	89.11±3.31 ^{ax}	74.63±6.64 ^{ay}	51.11±5.84 ^{az}
Dried 12h	72.80±5.22 ^{bw}	48.77±5.26 ^{bx}	17.71±3.02 ^{by}	1.93±0.72 ^{bz}
Dried 24h	94.05±4.03 ^{cw}	41.19±5.8 ^{cx}	22.88±7.57 ^{cy}	12.16±0.18 ^{cz}
Boiled 80 °C 40 min	6.45±2.00 ^{dw}	5.46±1.23 ^{dx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Boiled 100 °C 35 min	34.74±1.52 ^{ew}	5.03±5.27 ^{ex}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 12h and boiled 80 °C 30 min	22.94±2.34 ^{fw}	22.92±1.56 ^{fx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 12h and boiled 100 °C 25 min	23.09±1.20 ^{gw}	12.19±1.15 ^{gx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 80 °C 30 min	24.21±1.60 ^{hw}	10.21±1.07 ^{hx}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Dried 24h and boiled 100 °C 20 min	34.12±3.24 ^{iw}	20.98±3.24 ^{ix}	0.00±0.00 ^{dy}	0.00±0.00 ^{dy}
Steamed 45 min	95.32±2.99 ^{iw}	85.66±1.58 ^{jx}	83.45±1.89 ^{ey}	77.54±3.45 ^{ex}
Dried 12h and steamed 50 min	44.14±4.54 ^{kw}	34.21±0.98 ^{kx}	27.89±2.14 ^{fy}	19.78±2.54 ^{fz}
Dried 24h and steamed 50 min	43.25±1.24 ^{lw}	36.47±0.16 ^{lx}	28.77±2.12 ^{gy}	19.10±2.88 ^{gz}
Microwaved 450w - 30 s	93.44±5.48 ^{mw}	70.25±7.30 ^{mx}	46.73±3.56 ^{hy}	33.16±2.31 ^{hz}
Microwaved 900w - 20 s	95.73±4.84 ^{nw}	70.21±1.77 ^{mx}	46.85±5.84 ^{iy}	37.73±4.11 ^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-n) differ significantly ($P < 0.05$).

Means within each row with different letters (w-z) differ significantly ($P < 0.05$).

The antimicrobial activity of microwaved extracts ranged from strong to weak (95.73 to 33.16%). In the present study, extracts from fresh seaweeds had strong

antimicrobial activity at a concentration as low as 3.93 mg/ml. In the majority of reports on antimicrobial activities of seaweed extracts, bacterial growth inhibiting activities were investigated using standard agar disc diffusion assays (Bansemir *et al.*, 2006; Kuda *et al.*, 2007; Shanmughapriya *et al.*, 2008). There have been few quantitative reports on antimicrobial activity of seaweed extracts, however from those available; the results of the present study have been shown to be more effective than reported by Dubber and Harder (2008). These authors found and reported extracts of *Ceramium rebrum* and *Laminaria digitata* to have strong activity at 10 and 31 mg/ml, respectively, which is less potent than those of fresh seaweeds in the present study.

5.4 Conclusion

The findings of the present chapter indicate that the method of processing significantly influences the concentrations of phytochemicals, antioxidant and antimicrobial parameters in *H. elongata*. Consumption of *H. elongata* is dependent on some heat treatment in order to achieve an edible texture. Since heat processing invariably leads to a loss of antioxidant properties, a compromise must be reached between palatability and nutrition. It was found that a combination of drying followed by boiling reduced processing time and led to less leaching of phytochemicals. In terms of antioxidant activity of extract, a drying pre-treatment followed by boiling enhanced the phenolic content per gram of extract and as a result less amount of the extract would be required to have a significant effect in food products. Processing significantly affects the antimicrobial activity of extracts from *H. elongata*. Extracts from fresh *H. elongata* had the highest antimicrobial activity against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* with good

inhibition as low as 4.16 mg/ml extract. A better knowledge of how these processing conditions affects the phytochemical compounds of interest is of pivotal importance. Reduction in the moisture content and processing time also could have benefits in reducing transport and energy costs. Losses of health-related phytochemicals are thus likely to be a function of drying and processing parameters such as time, temperature and degree of wounding stress to the plant during these processes.

CHAPTER 6

QUALITY CHARACTERISTICS OF *H. ELONGATA* DURING DEHYDRATION AND REHYDRATION

*Moisture content, phytochemical content, texture and colour of *H. elongata* during
drying and rehydration*

*The results from this chapter were published as a peer-reviewed article in the LWT –
Food Science and Technology Journal*

Summary

Thermal processing if not properly monitored and controlled, can result in quality degradations. As seaweed is commonly preserved by drying, this chapter is focused on investigating the effect of a range of dehydration (25, 30, 35 and 40 °C) and rehydration temperatures (20, 40, 60, 80 and 100 °C) on the moisture content, phytochemical constituents, texture and colour of *H. elongata*. The dehydration kinetic study was complemented with the modelling of the terms of Fick's diffusion equation, for estimation of the diffusion coefficients. The diffusivity coefficient increased with heating from 5.6 to $12.2 \times 10^{-7} \text{ m}^2/\text{s}$, for the range of temperatures studied with estimated activation energy of 37.2 kJ/mol . The dehydration experimental data was also fitted to different empirical kinetic models, Newton, Logarithmic and Henderson-Pabis, and the goodness of fit for the different models was evaluated using the linear regression coefficient (R^2), sum square error (SSE), root mean square error (RMSE) and Chi-square statistic (χ^2). In terms of phytochemical content; drying at 25 °C resulted in a 31, 49 and 51% reduction in the total condensed tannin, total phenol and total flavonoid contents, respectively, as compared to fresh seaweed. However, the reduction seemed to decline as the drying temperatures were increased. The scavenging effect on DPPH radical was also greater for the fresh seaweed as compared to the dried form. However, an important increase in the phytochemical content was seen for higher temperatures (35 and 40 °C) when the moisture content reduced by 50% indicating that this semi-dry state is even more nutritious than the fresh form. Modelling of the moisture content of rehydration data was carried out by fitting to empirical kinetic models; Weibull, Peleg's, first-order and exponential association. Activation energies of 4.03, 4.28 and 3.90 kJ/mol were obtained for the parameters of Peleg's, first-order and exponential

models, respectively. Although restoration of the product to its original moisture content was achieved, rehydration resulted in losses in phytochemical content. Moisture equilibrium was obtained fastest at 100 °C (40 min) with losses of 83.2, 92 and 93 % in the total phenol, condensed tannin and flavonoid contents, respectively. There was a significant change in the colour of seaweed during both drying and rehydration, as compared to fresh ($P < 0.05$). Modelling of texture kinetics was investigated using zero (rehydration) and first (dehydration) order models. From the slopes, activation energies of 0.0157 and 0.0058 J/mol were obtained for the parameters of zero- and first-order models, respectively.

6.1 Introduction

Dehydration is one of the most important and oldest preservation methods employed for storage of foods. Water is removed from the food, reducing potential for microbial growth and undesirable chemical reactions (e.g. enzymatic browning) therefore increasing its shelf life. Plant products are often preserved by drying, however, enzymatic and/or non-enzymatic processes that may occur during drying of the fresh plant tissues may lead to significant changes in the composition of phytochemicals (Capecka *et al.*, 2005). Generally these processes cause negative attribute to the final food product, but studies by Nicoli *et al.*, (1999) showed that the overall antioxidant properties of certain foods may instead be enhanced due to improvement in the antioxidant properties of naturally occurring antioxidants and the formation of Maillard reaction products.

Being marine in nature, seaweeds contain a large amount of water, 75 - 85 % and as they are perishable in their fresh state and can deteriorate within a few days of

harvest; drying is an essential step before they can be used in industrial processing (Gupta *et al.*, 2011). The traditional way to preserve these plant products is by sun drying which is inconsistent as atmospheric conditions can vary greatly depending on the season. The dehydration process of seaweed in Ireland has not been optimised and traditional drying methods can lead to phytochemical losses. It was outlined in the 2011 Bord Iscaigh Mhara (BIM) *A Market Analysis towards the Further Development of Seaweed Aquaculture in Ireland* report that the seaweed industry requires input of technology such as specialist driers to improve the traditional drying methods. Drying helps to retard microbial growth, reduce the bulk handling thereby facilitating transportation and allows their use during the off-season (Mota *et al.*, 2009). However, other processes, promoted by high temperatures, can occur simultaneously with moisture removal during drying, resulting in undesirable alterations of certain characteristics of the material, such as shrinkage and colour changes (enzymatic and non-enzymatic browning) (Maskan, 2001).

Dehydrated food products are usually rehydrated before consumption. Rehydration is a complex process intended to restore the properties of the fresh product by contacting dehydrated products with a liquid phase. Typically, a higher rate of water absorption is observed during initial stages which then decline until equilibrium is achieved. Water temperature is the most important factor influencing the rehydration and generally more rapid rehydration is obtained at higher water temperatures. Treatments such as drying and rehydration produce changes in the structure and composition of product tissues. Rehydration or blanching is carried out to make vegetables more palatable but studies have shown that phenolic compounds are sensitive to heat, whereby blanching and boiling of vegetables for few minutes could

cause a significant loss of phenolic content which can leach into boiling water (Amin *et al.*, 2006). Since seaweeds are most commonly consumed after rehydration, it is important to investigate the effect of rehydration on the phytochemicals which are present.

Mathematical models of dehydration and rehydration are important to the design and optimisation of these processing conditions. Empirical equations frequently used to model the drying kinetics of food include: Newton, Page, Henderson–Pabis, Page modified, Logarithmic, Two-terms exponential, Thomson, Diffusion approach, Verma, Wang and Singh, Henderson-Pabis modified models and others (Vega *et al.*, 2007; Simal *et al.*, 2005). While models used for rehydration kinetics of foods include Weibull, Peleg's, first order and exponential association models (Peleg, 1988; Krokida and Marinos-Kouris, 2003; Sacchetti *et al.*, 2003). The study of rehydration kinetics helps in process optimisation and gives valuable information about the effect of process variables.

The main aims of this chapter were as follows:

1. To study the drying kinetics of hot-air drying of *H. elongata* at a range of temperatures (25, 30, 35 and 40 °C) which are typical temperatures applied in the seaweed industry.
2. To establish the rehydration kinetics of *H. elongata* at a range of temperatures (20, 40, 60, 80 and 100 °C).
3. To evaluate the effect of dehydration and rehydration conditions on the phytochemical content, texture and colour of *H. elongata*.

6.2 Materials and methods

6.2.1 Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. Samples were collected in January 2010 for the dehydration study and in August 2010 for the rehydration work. The seaweed was washed thoroughly with freshwater to remove epiphytes and salts and stored at -18 °C until analysis.

6.2.2 Dehydration procedure

Dehydration was carried out as described in section 3.12.1.

6.2.3 Rehydration procedure

Rehydration was carried out as described in section 3.12.2.

6.2.4 Extraction of phytochemicals

Seaweed samples (original 5 g fw) were powdered in liquid nitrogen using a mortar and pestle, then extracted as described in section 3.2.

6.2.5 Total phenolic content

The total phenolic content of seaweed samples was measured using the Folin-Ciocalteu method (Taga *et al.*, 1984) as described in section 3.4. The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram dry basis (db) (mg GAE/100 g db).

6.2.6 Total flavonoid content

Total flavonoid content was determined as described in section 3.5. Results were expressed as mg quercetin (QE)/100 gram db (mg QE/100 g db).

6.2.7 Total condensed tannins

Total condensed tannin content was determined as described in section 3.6. Results were expressed as mg catechin equivalents (CE)/100 gram db (mg CE/100g db) for whole seaweeds.

6.2.8 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as described in section 3.7. Results were expressed as % reduction in DPPH radical scavenging activity.

6.2.9 Texture evaluation

At specified experimental time seaweed samples (original 5 g fw) were removed to undergo instrumental texture analysis. Shear tests were performed as described in section 3.9.

6.2.10 Colour measurement

At specified experimental time seaweed samples (original 5 g fw) were removed to undergo colour analysis using a colourimetry (CIE Lab ColourQuest XE, Hunter Associates, Reston, VA, USA) as described in section 3.10.

6.2.11 Scanning electron microscopy

The structure of the seaweed samples (fresh, dried and rehydrated) were obtained with a scanning electron microscope as described in section 3.11

6.2.12 Drying kinetics expressed in terms of empirical models

The data obtained experimentally for the four different temperatures studied (25, 30, 35 and 40 °C) was plotted in the form of the dimensionless variable moisture ratio (MR) versus time (h) as described in section 3.13.1.

6.2.13 Estimation of Diffusion co-efficient

The estimation of the diffusion co-efficient was carried out as described in section 3.13.2.

6.2.14 Rehydration kinetics expressed in terms of empirical models

The data obtained experimentally for the five different temperatures studied (20, 40, 60, 80 and 100 °C) were plotted in the form of the dimensionless variable rehydration ratio (RR) versus time (min) as described in section 3.13.3.

6.2.15 Dehydration and rehydration texture modelling

Zero and first-order equations were used to describe the texture changes in *H. elongata* over the dehydration and rehydration process as described in section 3.13.4.

6.2.16 Statistical analysis

All experiments were performed in triplicate and replicated twice. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA) as described in section 3.23.

6.3 Results and Discussion

6.3.1 Effect of dehydration conditions on moisture content

Moisture content of fresh *H. elongata* was approximately 4.05 ± 0.05 kg water/kg dry matter. Fig. 6.1 shows the variation of moisture content as a function of time at the four dehydration temperatures studied (25, 30, 35 and 40 °C). All the drying curves show a clear exponential tendency and, as expected, an increase in the temperature accelerated the drying process. At 25 °C the drying rate became minimal and approached equilibrium after 8 h whereas equilibrium at 40 °C was attained after 5 h, representing 37.5% reduction in the total drying time. In addition, as the temperature increased, there was an increase in the rate of mass transfer (water) to achieve similar equilibrium moisture content (approximately 0.98 g water/100 g db).

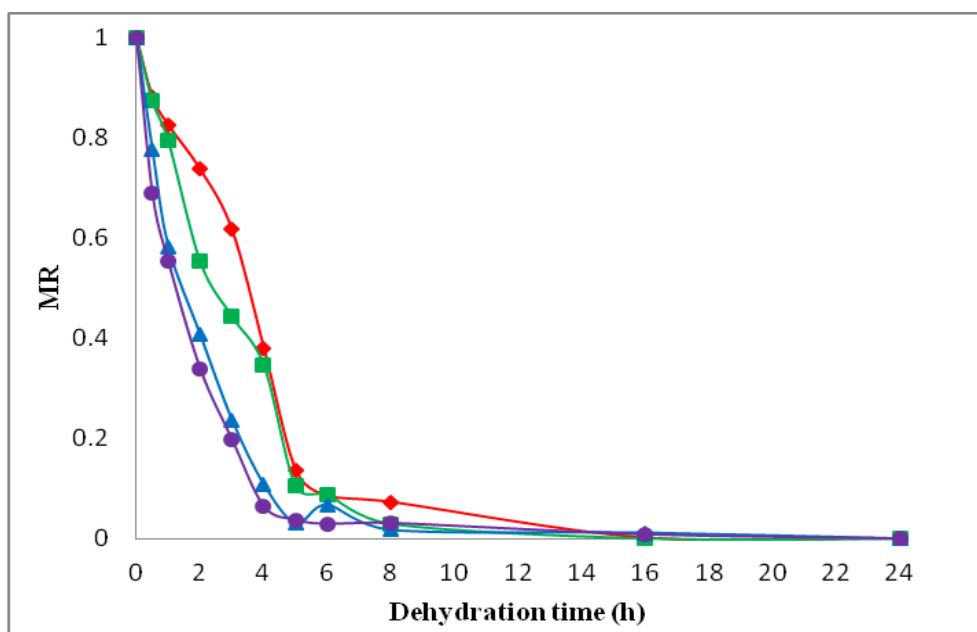


Fig. 6.1 Experimental drying curves of *H. elongata* at different temperatures (♦ : 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C)
Each value is presented as mean \pm SD (n = 6).

Seaweeds have a high rate of moisture loss under atmospheric conditions and generally, the seaweed industry employs outdoor drying under atmospheric conditions. Therefore, the dehydration temperatures applied in the present study were on the lower side than typical drying temperatures of food products in order to simulate the air drying conditions in the Irish seaweed industry. At the same time, drying was carried out under controlled conditions to achieve optimum drying time which will be short and will not reduce the final quality of the dried product. Moreover, preliminary experiments had shown that drying seaweeds at temperatures above 50 °C resulted in colour darkening within two hours with a large loss in the antioxidant properties. The percentage reduction of antioxidants for the samples dried at 50 °C was 87%.

6.3.2 Estimation of diffusion co-efficients

The traditional method for studying the mass transfer at a non-stationary state for the drying of foodstuffs is the Fick's equation (Eq. 3.8), from which the effective diffusivity coefficient (D_{eff}) is determined. Effective diffusivities of hot air dried seaweeds at different temperatures were obtained from the gradient of the plots of $\ln(MR)$ versus drying time (t) (Eq. 3.9) for 25, 30, 35 and 40 °C with slopes of 0.3483 h⁻¹, 0.4841 h⁻¹, 0.5224 h⁻¹ and 0.6566 h⁻¹, respectively. Table 6.1 shows the results of the fitting to Eq. 3.9, which allowed the calculation of the values of the diffusion coefficients, D_{eff} , at the different temperatures by Eq. 3.10. The values of the R^2 varied from 0.9262 - 0.9868 at the studied temperature range, and the diffusivity increased with temperature from a minimum of 5.6×10^{-7} m²/s at the lowest temperature to a maximum of 12.2×10^{-7} m²/s for the highest temperature.

Table 6.1 Estimation of diffusion co-efficients for seaweeds drying between 25 - 40 °C

	25 °C	30 °C	35 °C	40 °C
D_e (m ² /s)	5.6×10^{-07}	7.8×10^{-07}	8.5×10^{-07}	12.2×10^{-07}
L (m)	0.002	0.002	0.002	0.002
R^2	0.9262	0.9868	0.9419	0.9822

Several authors have shown similar behaviour of D_{eff} when working with okra (Doymaz, 2005), aloe vera (Vega *et al.*, 2007) and onions (Mota *et al.*, 2009). However, the diffusivity values obtained in the present chapter are higher than those reported in literature for other vegetables (Chong *et al.*, 2008; Vega *et al.*, 2007). The reason for this could be the higher initial water content of *H. elongata* allowing greater diffusion coefficients, since the process of diffusion is favoured in products

with higher proportions of water and lower proportions of solids (Guiné and Fernandes, 2006). Diffusivity values were then used to fit Eq. 3.12, to estimate the values of the diffusivity for an infinite temperature, D_o , and the activation energy for moisture diffusion, E_a . The results of this fitting are presented in Fig. 6.2 and they show a high quality fitting with R^2 value of 0.9484. The value obtained for the diffusion coefficient at an infinite temperature, D_o , was $1.87 \text{ m}^2/\text{s}$, with activation energy for moisture diffusion, E_a , of 37.2 kJ/mol .

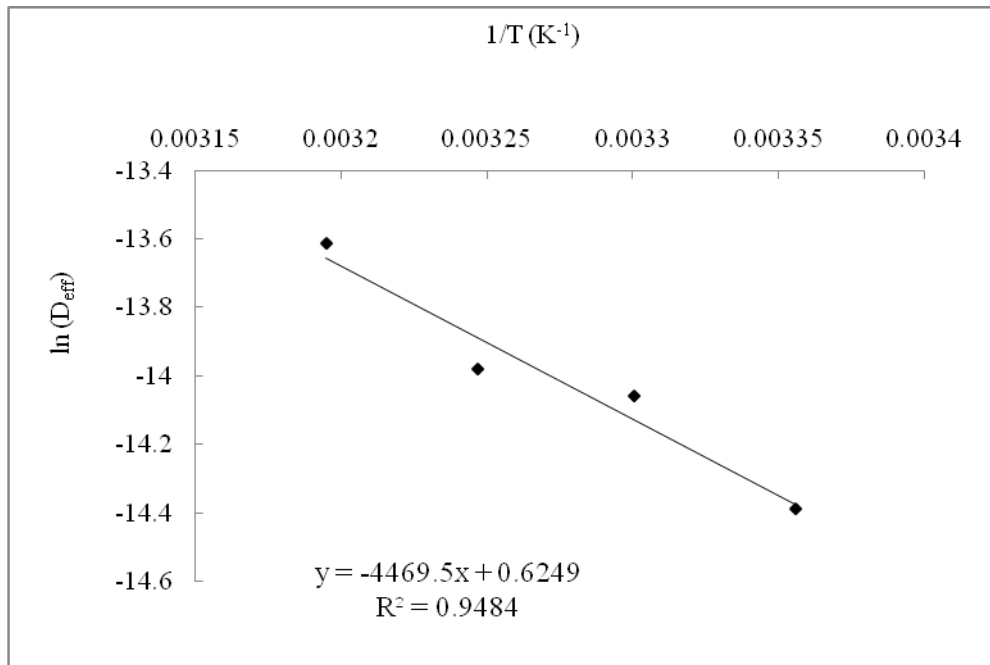


Fig. 6.2 Variation of effective diffusivity (m^2/s) with temperature

6.3.3 Modelling of dehydration curves

Although the dehydration kinetics was temperature-dependent, the differences in the moisture content decreased as the system reached equilibrium. The drying kinetics data obtained for the four temperatures studied was fitted to three different empirical kinetic models commonly cited in the literature as shown in Table 6.2.

Table 6.2 Empirical models used for the fitting of drying kinetics of *H. elongata* at different temperatures

Model name	Equation
Newton	$MR = \exp (-kt)$
Logarithmic	$MR = a \exp (-kt) + c$
Henderson-Pabis	$MR = a*\exp (-kt)$

The values of the estimated parameters with the standard error obtained for each model are presented in Table 6.3 and the values of the different statistical parameters can be seen in Table 6.4. It was observed that R^2 values ranged from 0.948 to 0.995 for the different models. The fact that drying kinetics was temperature dependent could be ascertained from the fact that the value of the parameter ' k ' increased for all the models as the drying temperature increased.

Table 6.3 Results of fitting drying kinetics to the Newton, Logarithmic model and the Henderson-Pabis

(values in brackets are the standard error for the parameters)

Model		25 °C	30 °C	35 °C	40 °C
Newton	<i>k</i>	0.254 (±0.03)	0.313 (±0.02)	0.504 (±0.02)	0.591 (±0.017)
Logarithmic	<i>a</i>	1.103 (±0.089)	1.065 (±0.05)	1.0 (±0.025)	0.976 (±0.025)
	<i>k</i>	0.247 (±0.048)	0.305 (±0.03)	0.499 (±0.032)	0.581 (±0.037)
	<i>c</i>	-0.05 (±0.07)	-0.03 (±0.04)	-0.002 (±0.015)	0.003 (±0.01)
Henderson-Pabis	<i>a</i>	1.058 (±0.067)	1.038 (±0.04)	0.998 (±0.057)	0.978 (±0.02)
	<i>k</i>	0.271 (±0.036)	0.326 (±0.026)	0.503 (±0.02)	0.576 (±0.03)

****k*, *a* and *c* are model parameters**

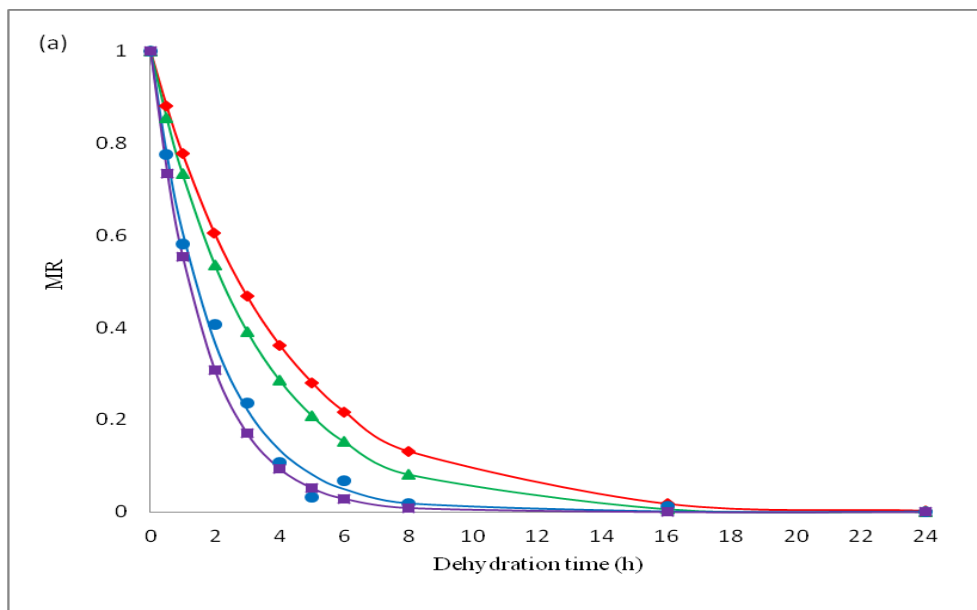
Table 6.4 Statistical indices upon modelling the drying of *H. elongata* at a range of drying temperatures

Model	Temperature (°C)	SSE	RMSE	χ^2	R^2
Newton	25	0.008	0.088	0.009	0.9434
	30	0.003	0.051	0.003	0.9794
	35	0.0005	0.023	0.0006	0.9794
	40	0.0006	0.024	0.0006	0.9943
Logarithmic	25	0.007	0.082	0.009	0.9515
	30	0.002	0.046	0.003	0.9832
	35	0.0005	0.023	0.0007	0.995
	40	0.0005	0.023	0.0007	0.9948
Henderson-Pabis	25	0.007	0.085	0.009	0.948
	30	0.002	0.049	0.003	0.9814
	35	0.0005	0.023	0.0007	0.995
	40	0.0005	0.023	0.0006	0.9948

Fig. 6.3 shows the predicted and experimental points obtained for the four temperatures with the different models tested; Newton (a), Logarithmic (b) and Henderson-Pabis (c). The results verified that the three models used in this study show a good predicting capacity, and revealed good performance for the temperatures tested, over the entire duration of the drying process. However, Newton model had the lowest R^2 values among all the three models. The values of the standard error of the parameter ' k ' in the Newton model vary between 3 - 12% for all the temperatures studied. The values of standard error for Henderson-Pabis model were within acceptable range for parameter ' a ' (2 - 6%) and ' k ' (5 - 13%). Regarding the Logarithmic model, the values for the standard errors of parameter ' a ' vary from 3 - 8% and ' k ' varies from 6 - 19% and are within the acceptable range, but the

standard errors of the parameters ' c ' for all the temperatures is of the same order of the value itself or greater.

In order to prove the dependence of parameter ' k ' on the drying temperature (Vega *et al.*, 2007), the Arrhenius equation was applied, graphically representing $\ln k$ versus $1/T$ (Simal *et al.*, 2005). Straight lines were obtained with regression coefficients (R^2) higher than 0.98 (Fig. 6.4), from whose slopes activation energies of 46.7, 47.4, and 41.8 kJ/mol were obtained for the parameters of Newton, Logarithmic and Henderson-Pabis model, respectively.



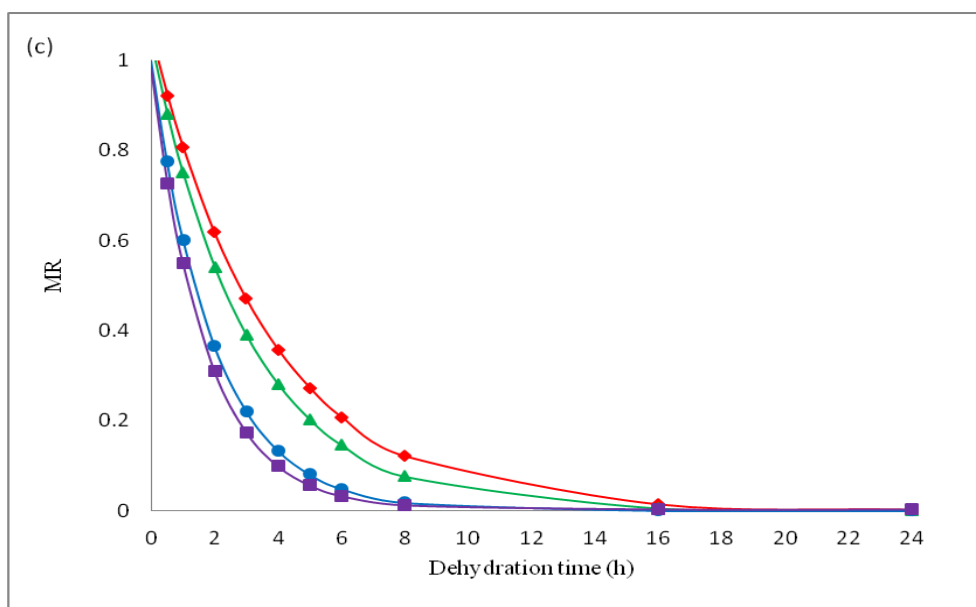
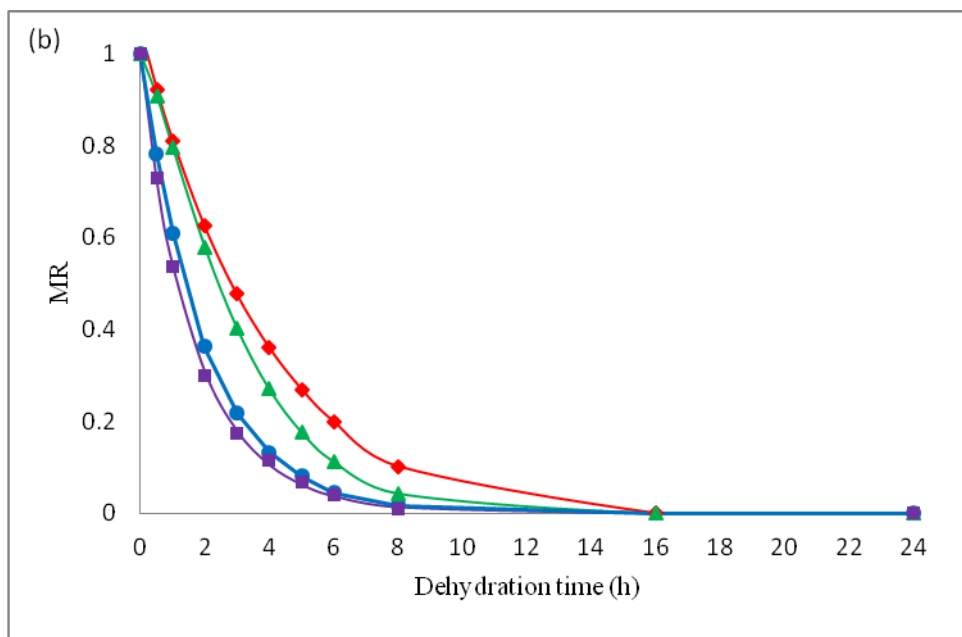


Fig. 6.3 Experimental and Predicted drying curves for (a) Newton (b) Logarithmic model (c) Henderson-Pabis model for the four working temperatures (♦ : 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C)

Points represent the experimental values and lines represent the predicted values.

Each value is presented as mean \pm SD (n = 6).

MR: moisture ratio

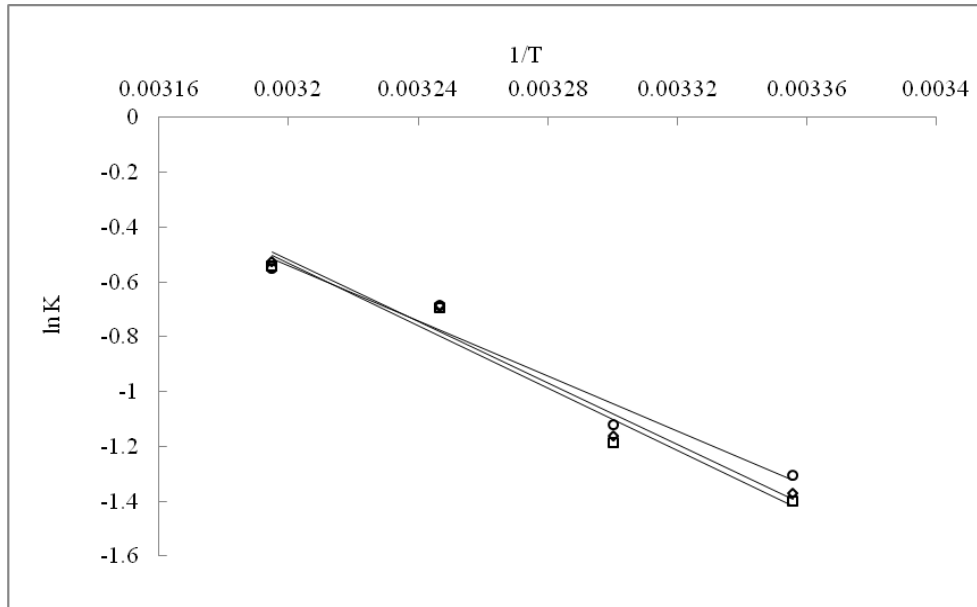


Fig. 6.4 Graphical representation of the influence of temperature on (k) upon the application of different drying models. $1/T$ (kJ/mol). Key: Δ ; Newton ($R^2 = 0.9494$); \square : Logarithmic ($R^2 = 0.9596$); \circ :Henderson-Pabis ($R^2 = 0.9598$)

Based on the similarities between the activation energy of the diffusivity coefficient (37.16 kJ/mol) and the parameter ' k ' for the studied models (46.7, 47.4, and 41.8 kJ/mol), in addition to the values reported by Senadeera *et al.* (2003) for other vegetables (12.87 - 58.15 kJ/mol), the parameter ' k ' can be considered as pseudodiffusivity. The parameter ' k ' represents a pseudodiffusional behaviour of matter transfer as stated in Fick's second law. The ANOVA carried out on the parameters ' a ' of Henderson-Pabis model showed no statistically significant difference ($P > 0.05$) of these parameters as related to temperature, suggesting they probably depend more on the characteristics of the tissue and/or the drying air flow. Vega *et al.* (2007) also reported similar observations for the parameter ' a ' of Henderson Pabis model.

6.3.4 Effect of dehydration on the phytochemical constituents

Food antioxidant activity depends on many factors such as the lipid composition, antioxidant concentration, temperature, oxygen pressure, and the presence of other antioxidants and many common food components, e.g. proteins and water. Processing of any kind will affect content, activity and bioavailability of bioactive compounds. With this fact in mind, the present chapter aimed at investigating the effect of different drying temperatures on the total phenol content, total flavonoid, total condensed tannin and DPPH content in the seaweed extract.

The TPC was monitored for *H. elongata* dried at different temperatures over the entire duration of dehydration time (Fig. 6.5). The initial TPC was 1.55 ± 0.026 g GAE/100 g dry seaweed. The content of total phenol was found to be higher than those reported for other common algae such as *Laminaria*, *Undaria*, *Scytosiphon*, *Tunbinaria* (Chandini *et al.*, 2008; Yuan and Walsh, 2006; Kuda *et al.*, 2005; Jiménez-Escrig *et al.*, 2001). Overall drying at different temperatures resulted in a reduction in the TPC, however the content was still higher than the values reported for dried seaweeds in literature (Kuda *et al.*, 2005). Wolfe and Liu (2003) also reported a high reduction of TPC in apple peels when compared with fresh peels. In the present chapter, drying at lower temperatures (25 and 30 °C) resulted in a continuous reduction of TPC although a small increase was seen (at 4 h) when the moisture content had reduced by half. For higher temperatures, 35 and 40 °C, an increase in the TPC content was seen for the first 2 h after which it started decreasing.

Maximum increase of 41% in the TPC was seen after drying at 40 °C for 2 h. Since identical amounts of sample were taken for fresh and dried seaweeds therefore, there was no influence of residual moisture on the antioxidant capacity or TPC of the samples. This increase could be related to the developmental changes and wound-like response due to dehydration. Dixon and Paiva (1995) reported that plants respond to wounding with increases of phenolic compounds involved in the repair of wound damage. However, at the end of the 24 h drying period a reduction (29 - 51%) in the TPC was seen for *H. elongata* dried at different temperatures.

Higher loss of TPC was observed at lower drying temperatures and there were significant differences in TPC in seaweed samples dried at different temperatures ($P < 0.05$). Maximum reduction of 51% in the TPC content was seen in *H. elongata* dried at 25 °C whereas a reduction of only 29% was seen when drying was carried out at 40 °C, as compared to fresh seaweed. A probable reason for this could be the long drying time of seaweeds at 25 °C to achieve a similar equilibrium moisture content as compared to when drying at 40 °C. Jiménez-Escrig *et al.* (2001) reported a 98% reduction in the TPC content in brown seaweed *Fucus* dried at 50 °C for 48 h. Garau *et al.* (2007) reported that longer drying times resulted in a reduction of TPC for orange by-products.

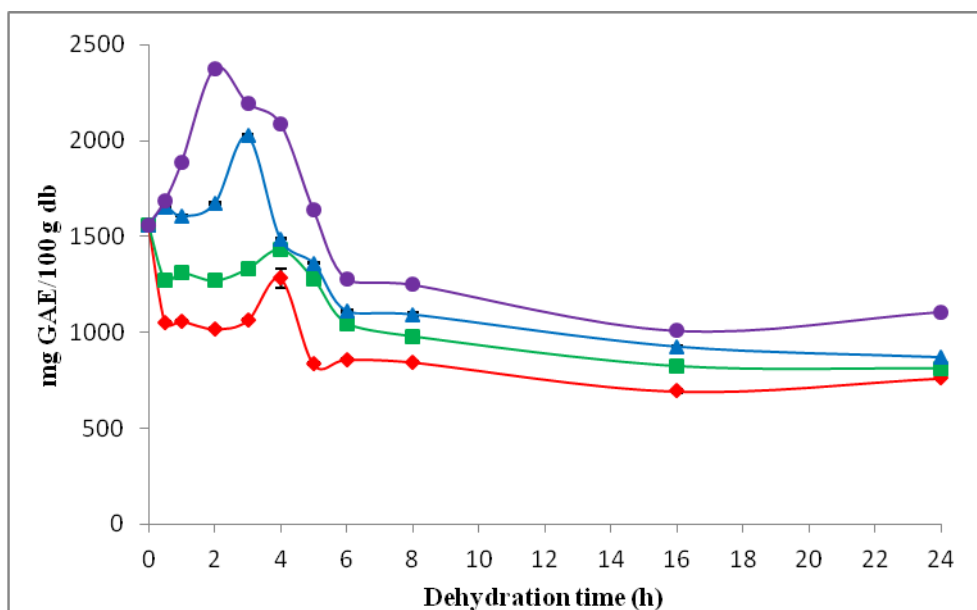


Fig. 6.5 Effect of drying temperatures on the total phenolic content of *H. elongata* (♦: 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C)

Each value is presented as mean \pm SD (n = 6).

Changes in the phenolic compositions and contents might also occur during drying and this might also be a reason for the reduction of TPC (Guan *et al.*, 2005). Also, the lower dehydration temperatures used in the present study may not inactivate the oxidative enzymes completely, which may have in turn resulted in some oxidation of the phenolic substances and resulted in a relatively lower phenolic content. Decreases in TPC during drying can also be attributed to the binding of polyphenols with other compounds (proteins) or the alterations in the chemical structure of polyphenols which cannot be extracted or determined by available methods (Qu *et al.*, 2010; Martín-Cabrejas *et al.*, 2009).

Fig. 6.6 shows the variation in the TFC content over the entire drying period for the four different temperatures studied. The TFC of the fresh seaweeds was $0.49 \text{ g} \pm$

0.019 QE/100 g dry seaweed. The TFC also reduced continuously for the lower temperatures but at higher temperatures it increased initially after which it decreased. Again the increase in the content was seen at a time when the moisture had reduced by 50%. Although drying led to a reduction in the TFC, the % reduction declined as the drying temperature increased. A percentage reduction of 49% and 30% were seen at 25 and 40 °C, respectively.

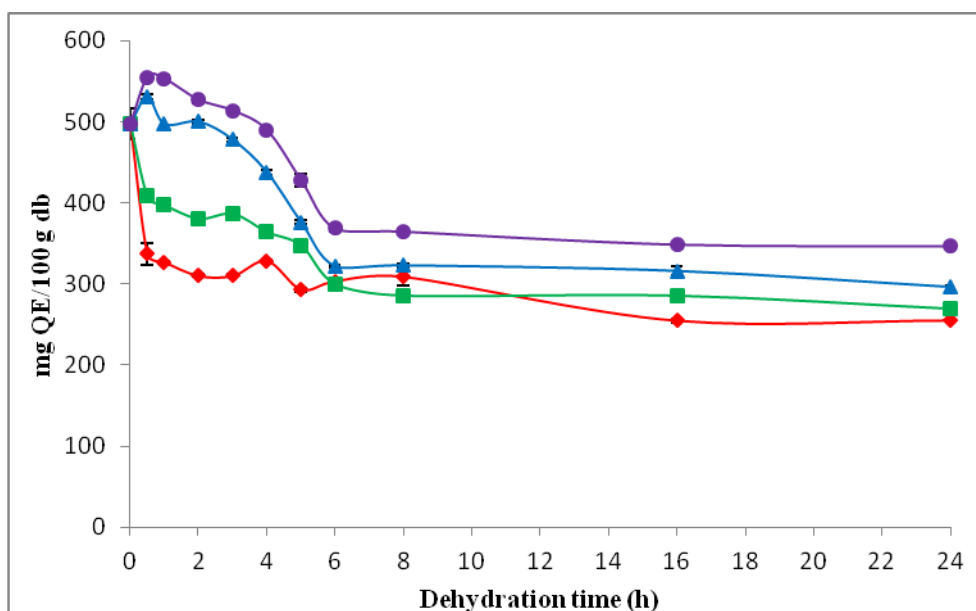


Fig. 6.6 Effect of drying temperatures on the total flavonoid content of *H. elongata* (♦: 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C)

Each value is presented as mean \pm SD (n = 6).

The total tannin content (TTC) of the dehydrated seaweed samples at the four temperatures studied can be seen in Fig. 6.7. At 25 and 30 °C, there was a similar trend in the behaviour of TTC where there was an initial decrease in the first hour after which the content became constant after 6 h. For example, the initial TTC of fresh seaweed was 0.43 ± 0.007 g CE/100 g db which decreased to 0.29 ± 0.004 g

CE/100 g db at 25 °C, and was 0.3 ± 0.003 g CE/100g db at the end of 24 h (30.9% decrease). However, similar to TPC, the TTC increased up to 0.59 ± 0.006 and 0.62 ± 0.004 g CE/100g db in the initial 3 h of dehydration at 35 and 40 °C, respectively, and then decreased. The TTC was highest in *H. elongata* dried at 40 °C; the content increased to 0.62 g CE/100 g db at 3 h then decreased to 0.48 g CE/100 g db at the end of the 24 h drying period but still represented a 12.3% increase as compared to the initial TTC content.

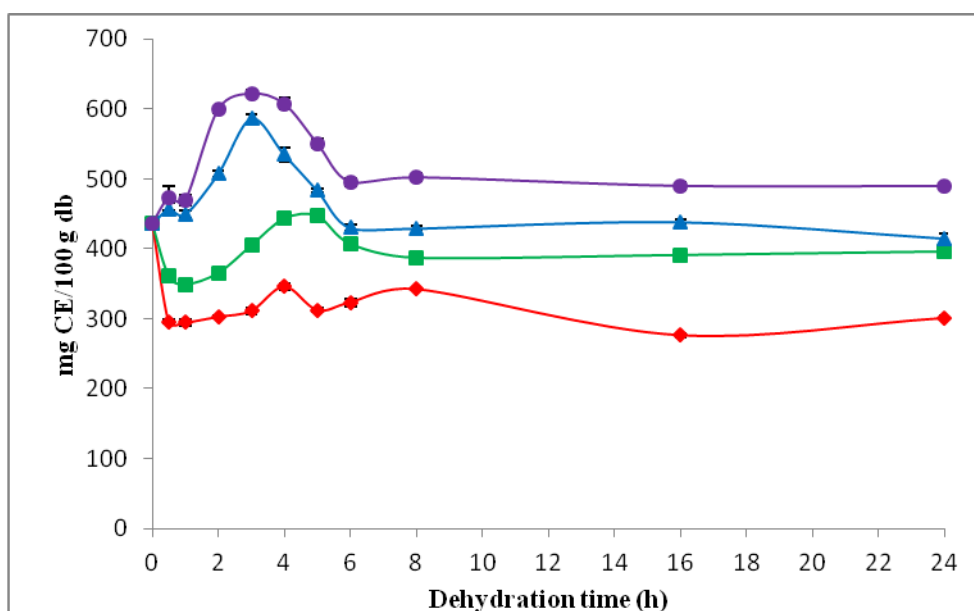


Fig. 6.7 Effect of dehydration temperatures on the total condensed tannin content of *H. elongata* (♦: 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C)

Each value is presented as mean \pm SD (n = 6).

Dehydration resulted in significant decrease in the antioxidant activity exhibited by the reduction in DPPH free radical scavenging activity tested at an extract concentration of 50 μ g/ml (Fig. 6.8). Fresh *H. elongata* had a DPPH radical scavenging activity of 78.9%. At the end of 24 h dehydration at 25 and 30 °C,

reductions of the magnitude of 17.3 and 12.8% were observed as compared to fresh seaweed. While at 35 and 40 °C reduction were 7.3 and 4.5%, respectively.

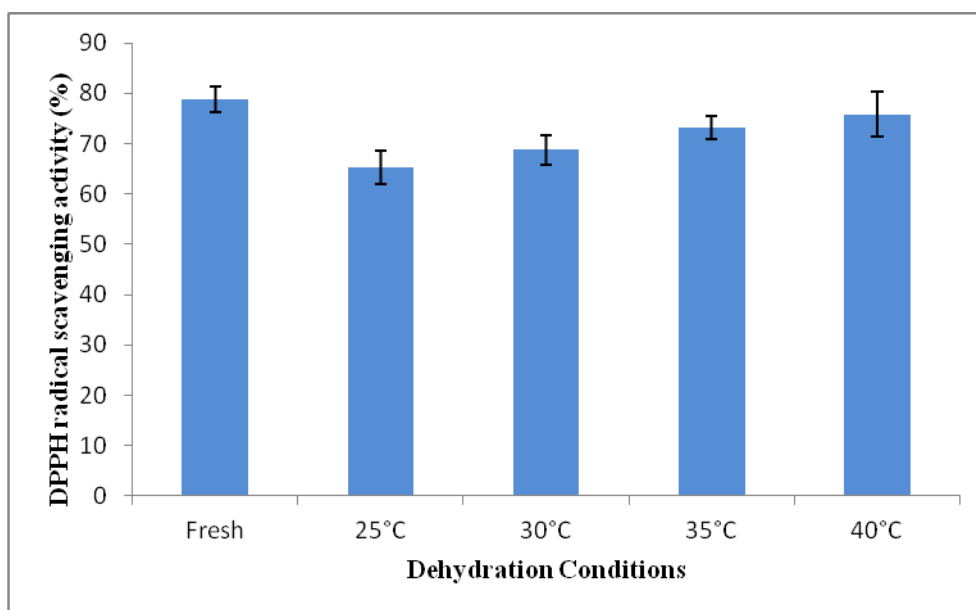


Fig. 6.8 Effect of dehydration temperatures on the DPPH radical scavenging activity of fresh *H. elongata*

Each value is presented as mean \pm SD (n = 6).

Li *et al.* (2006) had reported that a combination of high drying temperatures and long drying times might destroy some of the phenol compounds. In addition, all the plant cell components adhere together in the absence of water, and possibly making the extraction with solvent more difficult; as a result, the overall recoveries might be lower than expected (Li *et al.*, 2006). While fresh algal specimens have been reported to contain several hydrophilic, but labile, antioxidant molecules such as L-ascorbate (Aguilera *et al.*, 2002; Burritt *et al.*, 2002) and GSH (Burritt *et al.*, 2002), sun drying and subsequent storage of algae will considerably decrease the levels of these labile antioxidants (Burritt *et al.*, 2002; Jiménez-Escrig *et al.*, 2001). The drying process would generally result in a depletion of naturally occurring antioxidants in raw materials from plants. Intense and/or prolonged thermal

treatment may be responsible for a significant loss of natural antioxidants, as most of these compounds are relatively unstable (Lim and Murtijaya, 2007).

6.3.5 Effect of rehydration conditions on moisture content

As 40 °C demonstrated to be the optimal drying temperature in terms of phytochemical content, this drying temperature was also applied to the seaweed rehydration study. The dried seaweed samples were rehydrated at 20, 40, 60, 80 and 100 °C until the moisture content reached equilibrium (up to 80 min) in order to investigate the effect of temperature on rehydration kinetics. The initial moisture content of the fresh seaweed was 4.07 ± 0.02 g water/g db and the dried seaweed (40 °C for 24 h) contained 0.074 ± 0.01 g water/g db, representing a 98.1% reduction in water content/g db. Fig. 6.9 shows the variation of moisture content as a function of time for the five rehydration temperatures. All rehydration curves show a clear exponential tendency, and as expected, the rehydration time decreased when the temperature increased until all the samples reached similar equilibrium moisture content. As rehydration time progressed, there was a decrease in the driving force for water transfer and the system slowly attained equilibrium.

Higher temperatures resulted in an increase in the magnitude of absorbed water. For example at 20 °C, rehydration reached equilibrium at 75 min while equilibrium was reached after 65, 55 and 45 min at 40, 60 and 80 °C, respectively. Increasing the temperature to 100 °C increased the rate of water absorption and equilibrium was attained after 40 min, representing 46.6% reduction in the rehydration time as compared to rehydration at 20 °C.

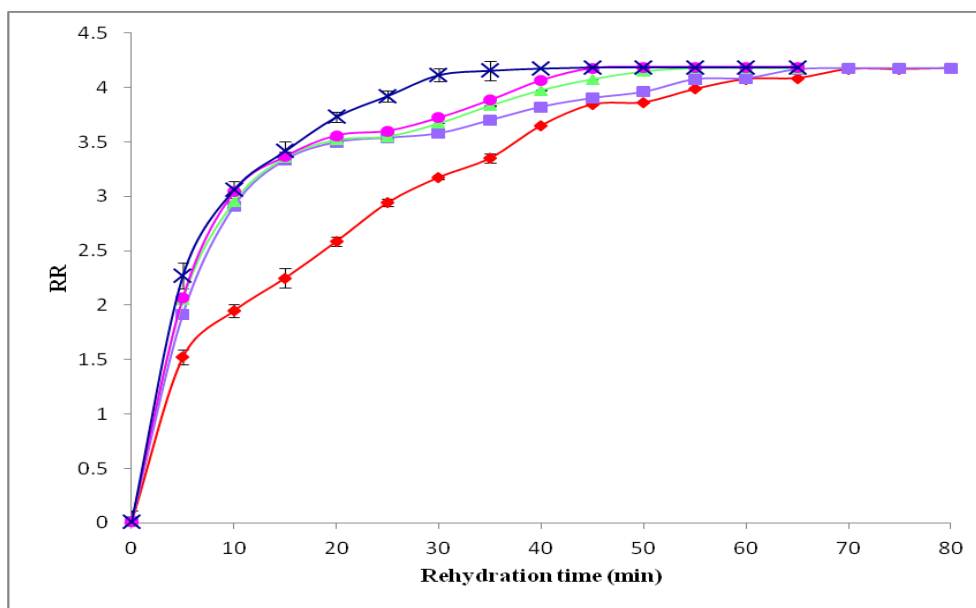


Fig. 6.9 Experimental rehydration curves of *H. elongata* at different temperatures (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C)

Each value is presented as mean \pm SD (n = 6).

It is generally accepted that the degree of rehydration is dependent on the degree of cellular and structural disruption. Often there can be irreversible cellular rupture and dislocation, resulting in loss of integrity and hence, a dense structure of collapsed, greatly shrunken capillaries with reduced hydrophilic properties can occur. This is reflected by the inability to imbibe sufficient water to rehydrate fully (Krokida and Marinos-Kouris, 2003). Seaweeds grow in distinct vertical bands on the seashore and it is well known that their ability to recover from physiological processes following desiccation is correlated to their shore position. Despite this, little is known of the cellular mechanisms by which intertidal seaweeds limit membrane damage during desiccation and subsequent rehydration. An ability to tolerate desiccation is therefore a prerequisite for their survival (Burritt *et al.*, 2002). The dried seaweed samples in the present chapter had the ability to rehydrate with final moisture contents equal or higher to that of the fresh seaweed. This indicates that the hydrophilic properties had

the ability to imbibe sufficient water at all of the working temperatures which is of great benefit. Not all plants have the ability to be dried and rehydrated to their original capacity, for example, Vega-Gálvez *et al.* (2009) found that aloe vera could only achieve a maximum rehydration capacity of 38% of the original product.

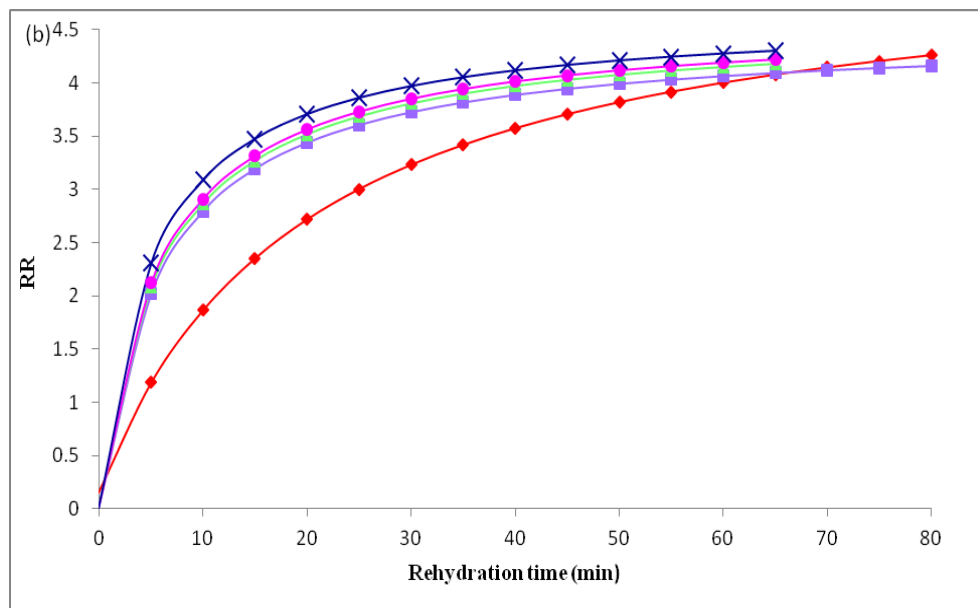
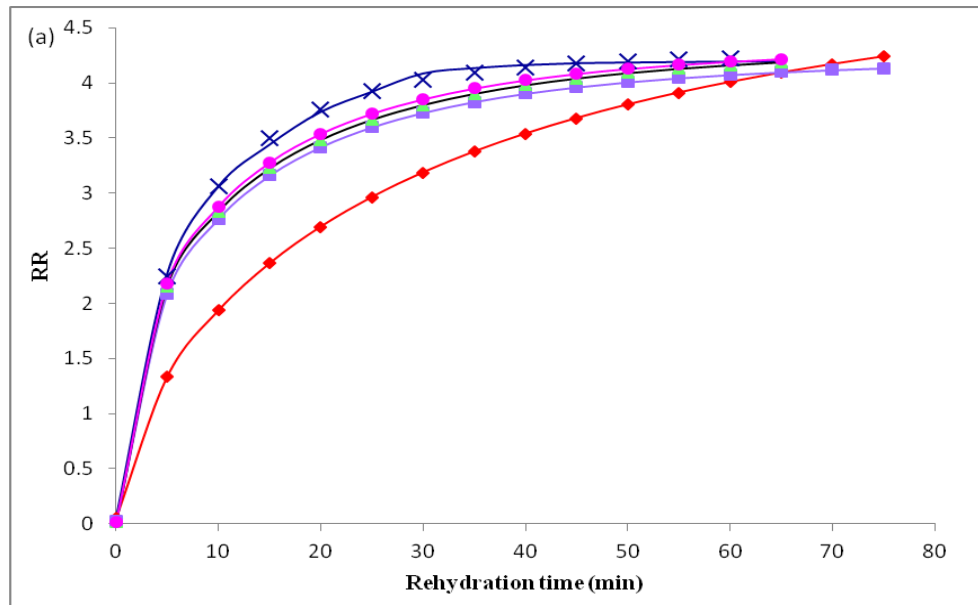
In order to describe the rehydration kinetics of seaweed, four empirical models; Weibull, Peleg's, first-order and exponential association were applied (Table 6.5).

Table 6.5 Empirical models used for the fitting of rehydration kinetics of *H. elongata* at different temperatures

Model name	Equation
Weibull	$RR = W_e + (W_o - W_e) \exp \left[-\left(\frac{t}{\beta}\right)^\alpha \right]$
Peleg	$RR = W_o + \frac{t}{k_1 + k_2 t}$
First-order rehydration kinetic model	$RR = W_e + (W_o - W_e) \exp(-k_{R1} t)$
Exponential association model	$RR = W_e [1 - \exp(-k_{R2} t)]$

All of the models applied provided a good agreement with the experimental data. The R^2 values (Table 6.6) ranged from 0.9807 to 0.9985 for the different models. Fig. 6.10 shows the predicted and experimental points obtained for the five temperatures for each of the four different models; Weibull (a), Peleg's (b), First-order (c) and Exponential (d). From the results obtained it can be verified that all four models applied in this study showed a good predicting capacity, and revealed good performance for the temperatures tested over the entire rehydration procedure.

First-order rehydration kinetic model had the lowest R^2 values among the four models.



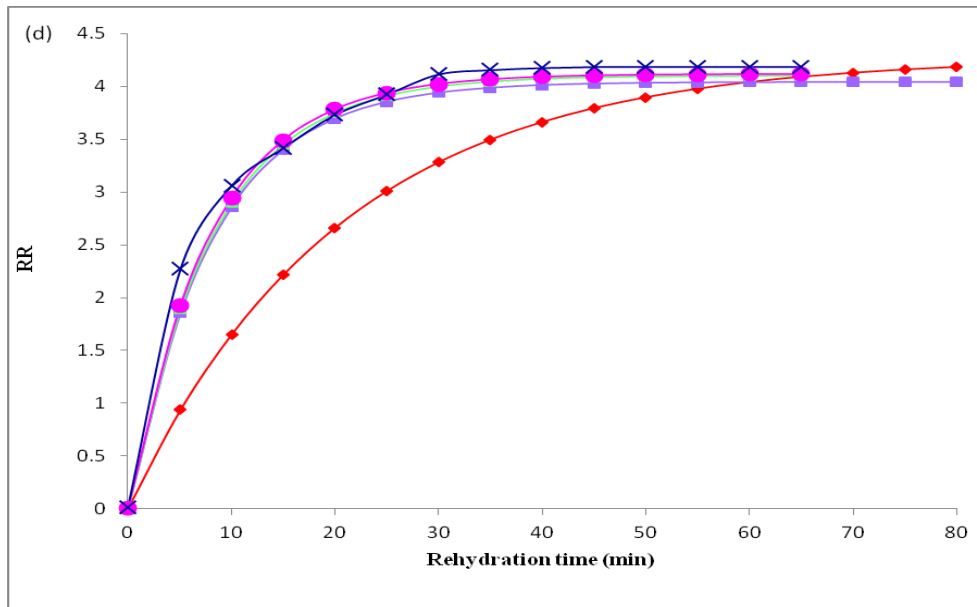
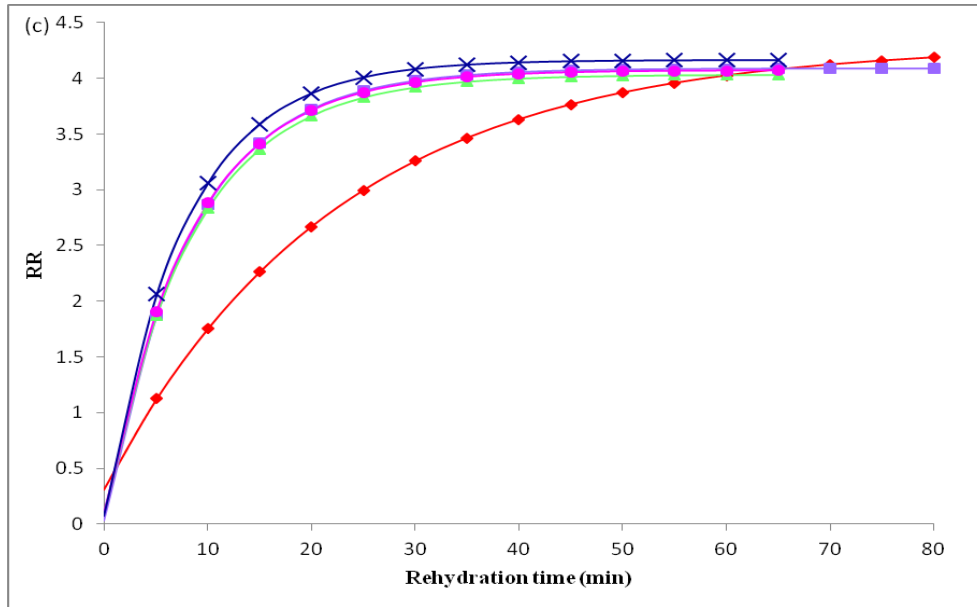


Fig. 6.10 Experimental and predicted rehydration curves for (a) Weibull (b) Peleg's (c) First order and (d) Exponential association model for the five working temperatures (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C) Points represent the experimental values and lines represent the predicted values.

Each value is presented as mean \pm SD (n = 6).

RR: Rehydration ratio

Table 6.6 Statistical indices upon modelling the rehydration of *H. elongata* at a range of temperatures

Model	Temperature (°C)	SSE	RMSE	χ^2	R^2
Weibull	20	0.01	0.1002	0.0106	0.9922
	40	0.0086	0.093	0.0091	0.9918
	60	0.0062	0.079	0.0067	0.9948
	80	0.0077	0.0882	0.0083	0.9936
	100	0.0018	0.0425	0.0019	0.9985
Peleg	20	0.0158	0.1257	0.0168	0.9878
	40	0.006	0.0775	0.006	0.9943
	60	0.005	0.0709	0.0054	0.9958
	80	0.0065	0.0806	0.007	0.9947
	100	0.0051	0.0714	0.0054	0.9959
First-order rehydration kinetic model	20	0.0216	0.1472	0.023	0.9833
	40	0.0315	0.7321	0.0335	0.9945
	60	0.0232	0.1525	0.025	0.9807
	80	0.0238	0.1545	0.0257	0.9806
	100	0.0086	0.0927	0.0092	0.9931
Exponential association model	20	0.0308	0.1755	0.0327	0.9936
	40	0.0269	0.1642	0.0286	0.9895
	60	0.0292	0.171	0.0022	0.9925
	80	0.0275	0.1659	0.0296	0.9907
	100	0.0092	0.0963	0.0099	0.9926

The examination of the parameters obtained from the models applied (Table 6.7) showed that the scale parameter (β) of the Weibull model, kinetic rate constant k_1 and characteristic constant k_2 of the Peleg's model are changing inversely proportional to the rehydration temperature applied. On the other hand, the kinetic constants of first-order (k_{R1}) and exponential association models (k_{R2}) are increasing with increased rehydration temperature. Parameter k_2 of the Peleg's model decreased as the temperature increased which was also reported by Vaga-Gálvez *et al.* (2009) for aloe vera. Solomon (2007) suggested that this parameter is related to maximum

capacity of water absorption or to equilibrium moisture content, in such a way that low values of k_2 show a higher water absorption capacity. Therefore the results show that water absorption capacity increases as water rehydration temperature increases. Similar trends were observed by Maskan (2002) and Moreira *et al.* (2008) for wheat and chestnuts. Moreover, time taken to reach equilibrium moisture content for the dried seaweed samples (We) decreased as the rehydration temperature increased which indicates that higher temperatures are more effective in reaching a maximum rehydration capacity in shorter times.

Table 6.7 Results of fitting of rehydration kinetics to the four models (values in curved brackets are the standard error and values in square brackets are 95 % confidence intervals)

Model	Parameter	20 °C	40 °C	60 °C	80 °C	100 °C
Weibull	<i>We</i>	5.027 (±0.445) [4.064-5.99]	4.224 (±0.094) [4.019-4.428]	4.335 (±0.131) [4.041-4.629]	4.336 (±0.129) [4.047-4.625]	4.25 (±0.311) [4.181-4.32]
	<i>Wo</i>	0.049 (±0.113) [-0.196-0.295]	-0.002 (±0.106) [-0.232-0.227]	0.005 (±0.093) [-0.203-0.213]	0.005 (±0.104) [-.0226-0.237]	0.017 (±0.05) [-0.094-0.129]
	α	0.671 (±0.076) [0.505-0.837]	0.638 (±0.066) [0.495-0.782]	0.62 (±0.066) [0.471-0.769]	0.636 (±0.073) [0.472-0.801]	0.766 (±0.037) [0.684-0.849]
	β	30.209 (±7.589) [13.814-46.605]	9.124 (±0.942) [7.025-11.224]	9.07 (±0.812) [7.316-10.824]	8.754 (±0.908) [6.73-10.777]	7.334 (±0.256) [6.762-7.906]
Peleg	<i>Wo</i>	0.163 (±0.126) [-0.107-0.435]	0.0044 (±0.084) [-0.179-0.182]	0.013 (±0.079) [-0.16-0.188]	0.01 (±0.09) [-0.187-0.209]	0.001 (±0.08) [-0.174-0.178]
	<i>k₁</i>	3.927 (±0.459) [2.942-4.913]	1.414 (± 0.102) [1.123-1.564]	1.312 (±0.096) [1.099-1.525]	1.27 (±0.105) [1.037-1.502]	1.079 (±0.079) [0.904-1.254]
	<i>k₂</i>	0.222 (±0.004) [0.213-0.233]	0.2198 (±0.004) [0.210-0.229]	0.218 (±0.004) [0.207-0.228]	0.215 (±0.004) [0.206-0.225]	0.195 (±0.006) [0.181-0.208]
First-order rehydration kinetic model	<i>We</i>	4.297 (±0.104) [4.073-4.522]	4.0269 (±0.037) [4.003-4.141]	4.032 (±0.064) [3.89-4.175]	4.071 (±0.065) [3.927-4.214]	4.163 (±0.037) [4.08-4.246]
	<i>Wo</i>	0.318 (±0.133) [0.031-0.605]	0.129 (±0.1) [-0.19-0.265]	0.116 (±0.164) [-0.246-0.48]	0.113 (±0.167) [-0.255-0.481]	0.095 (±0.101) [-0.127-0.317]
	<i>k_{R1}</i>	0.0447 (±0.004) [0.035-0.053]	0.103 (±0.011) [0.093-0.142]	0.119 (±0.011) [0.094-0.145]	0.12 (±0.006) [0.105-0.135]	0.13 (±0.007) [0.114-0.146]
Exponential association model	<i>We</i>	4.211 (±0.056) [4.147-4.392]	4.0175 (±0.04) [3.953-4.132]	4.024 (±0.048) [3.994-4.213]	4.063 (±0.048) [4.008-4.226]	4.159 (±0.036) [4.078-4.239]
	<i>k_{R2}</i>	0.0512 (±0.002) [0.043-0.053]	0.108 (±0.007) [0.106-0.138]	0.122 (±0.006) [0.106-0.137]	0.124 (±0.007) [0.108-0.141]	0.134 (±0.006) [0.119-0.148]

**We*, *Wo*, α , β , *k₁*, *k₂*, *k_{R1}* and *k_{R2}* are the model parameters

Table 6.7 also shows the values of parameters α and β of the Weibull model, where temperature has no linear influence on parameter α . However, parameter β decreases as temperature is increased. Similar behaviour was reported by Machado *et al.* (1998) and Cunningham *et al.* (2007) for puffed breakfast cereals and pasta, respectively. These authors suggested that parameter β represents the time needed to accomplish approximately 63% of the rehydration process.

Table 6.7 Statistical indices upon modelling the rehydration of *H. elongata* at a range of temperatures

Model	Temperature (°C)	SSE	RMSE	χ^2	R^2
Weibull	20	0.01	0.1002	0.0106	0.9922
	40	0.0086	0.093	0.0091	0.9918
	60	0.0062	0.079	0.0067	0.9948
	80	0.0077	0.0882	0.0083	0.9936
	100	0.0018	0.0425	0.0019	0.9985
Peleg	20	0.0158	0.1257	0.0168	0.9878
	40	0.006	0.0775	0.006	0.9943
	60	0.005	0.0709	0.0054	0.9958
	80	0.0065	0.0806	0.007	0.9947
	100	0.0051	0.0714	0.0054	0.9959
First-order rehydration kinetic model	20	0.0216	0.1472	0.023	0.9833
	40	0.0315	0.7321	0.0335	0.9945
	60	0.0232	0.1525	0.025	0.9807
	80	0.0238	0.1545	0.0257	0.9806
	100	0.0086	0.0927	0.0092	0.9931
Exponential association model	20	0.0308	0.1755	0.0327	0.9936
	40	0.0269	0.1642	0.0286	0.9895
	60	0.0292	0.171	0.0022	0.9925
	80	0.0275	0.1659	0.0296	0.9907
	100	0.0092	0.0963	0.0099	0.9926

6.3.6 Estimation of activation energy for kinetic constants

In order to prove the dependence of the k parameters; k_1 , k_{R1} and k_{R2} of the models, Peleg's, first-order and exponential on temperature, respectively, the Arrhenius equation was applied graphically represented by $\ln k$ versus $1/T$ (Simal *et al.*, 2005). Plots showed a linear relationship with regression coefficients (R^2) of 0.8889, 0.9314 and 0.9339 for Peleg's, first-order and exponential models, respectively, verifying the dependence of rehydration capacity on temperature. From the slopes, activation energies of 4.03, 4.28 and 3.90 kJ/mol were obtained for the parameters of Peleg's, first-order and exponential models, respectively. A graphical representation of the influence of temperature on the parameter (k_{R2}) of the exponential model can be seen in Fig. 6.11.

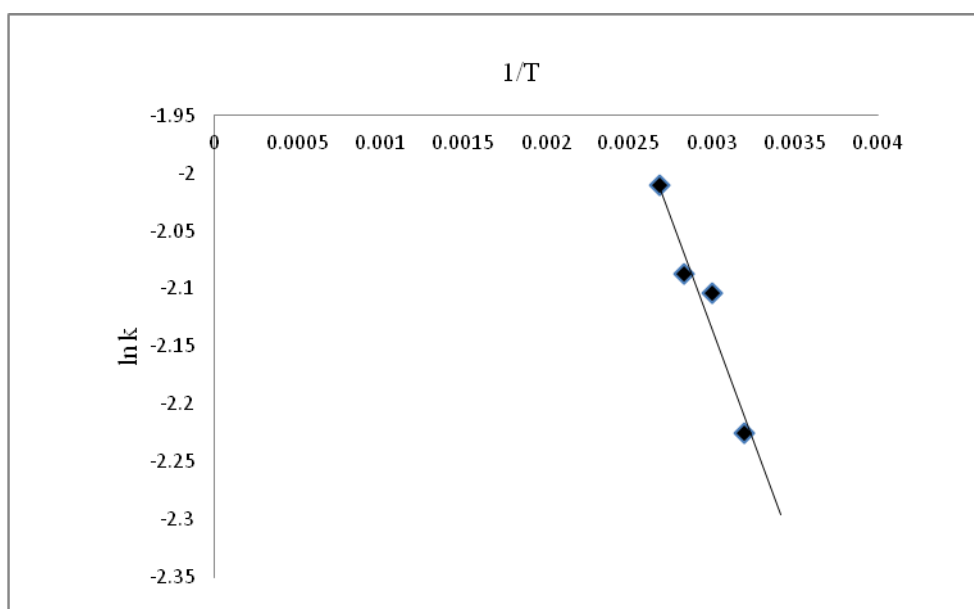


Fig. 6.11 Graphical representation of the influence of temperature on the parameter (k_{R2}) of the exponential model. $1/T$ (kJ/mol).

6.3.7 Effect of rehydration on the phytochemical constituents

Since seaweed would need to undergo some hydrothermal treatment in order to rehydrate prior to consumption, it was relevant to assess the effects of rehydration treatment on the stability of seaweed antioxidant properties. In chapter 5, it was illustrated that extracts from *H. elongata* have high antioxidant activity. Drying of *H. elongata* at 40 °C for 24 h results in a decrease in the phytochemicals up to 29%. The TPC was monitored for *H. elongata* rehydrated at different temperatures every 10 min over the duration of the rehydration process (Fig. 6.12). The initial content of TPC of the dried seaweed was 1.21 ± 0.02 g GAE/100 g db. Rehydration resulted in a steep decrease in TPC of dried seaweed within the first 10 minutes at each of the tested temperatures. As was previously discussed, rehydrating the dried seaweed at 100 °C increased the rate of water absorption and equilibrium moisture was attained after 40 min. At this time the TPC of the seaweed was 0.2 ± 0.009 g GAE/100 g db. Studies have shown that phenolic compounds are sensitive to heat, whereby boiling of vegetables for few minutes could cause a significant loss of phenolic content which can leach into blanching water (Amin *et al.*, 2006). Bunea *et al.* (2008) reported a 50% loss of TPC in spinach blanched for 10 min.

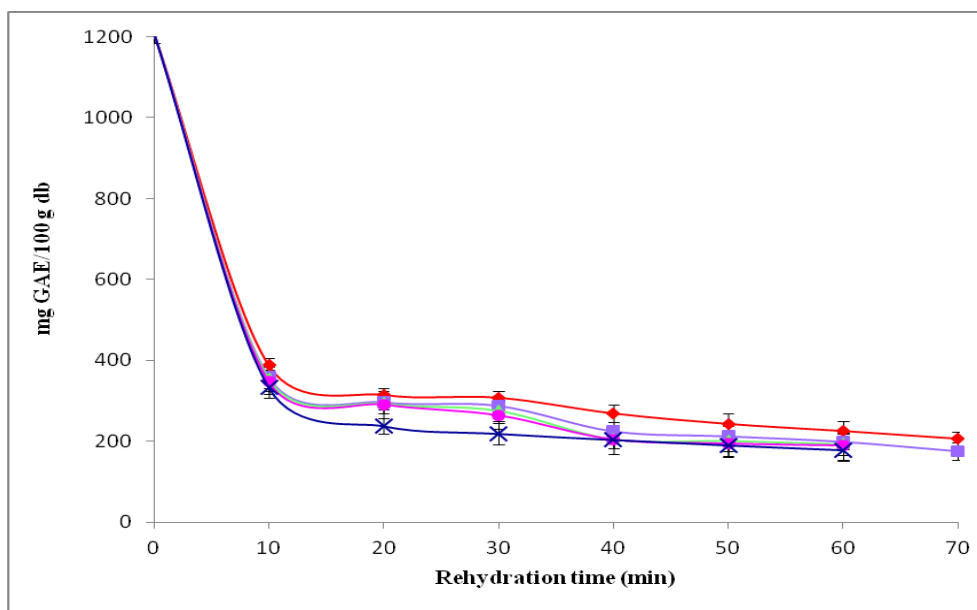


Fig. 6.12 Effect of rehydration temperatures on the total phenolic content of *H. elongata* (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C)
Each value is presented as mean \pm SD (n = 6).

Fig. 6.13 shows the variation in the TFC for the five temperatures studied. The TFC of the dried seaweeds was 0.49 ± 0.016 g QE/100 g db. Rehydration led to a reduction in TFC which was proportional to the increase in time and temperature. Reductions in the range of 88.3 - 93.2% in TFC were seen at 20, 40, 60, 80 and 100 °C, respectively at the end of 60 min of rehydration. The fastest equilibrium moisture content was reached at a temperature of 100 °C for 40 min with a 93% reduction in TFC. Release of flavonoids and increased chemical extraction of these compounds could be induced by the effect of boiling (Olivera *et al.*, 2008). This release of flavonoids coupled with contact and leaching into water could have resulted in high reduction in TFC for boiled samples. The results of the present chapter are similar to Olivera *et al.* (2008) who found that boiling decreased TFC in brussels sprouts.

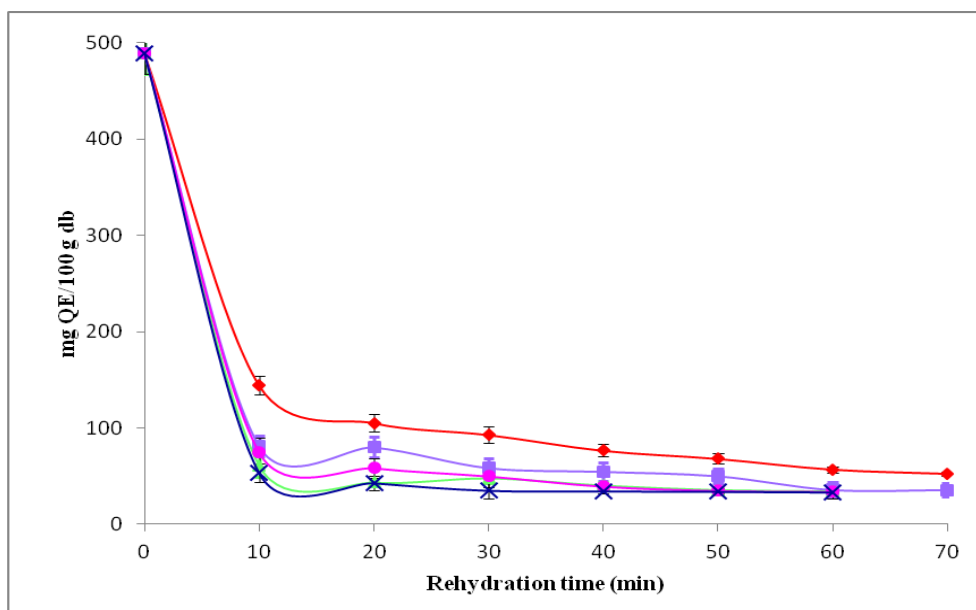


Fig. 6.13 Effect of rehydration temperatures on the total flavonoid content of *H. elongata* (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C)
Each value is presented as mean \pm SD (n = 6).

Fig. 6.14 shows the TTC of rehydrated dried seaweed for the five temperatures studied. Similar to TPC and TFC, rehydration led to a reduction in TTC which was proportional to the increase in time and temperature. Reductions in the range of 81.5 - 92.2% in TTC were seen between 20 and 100 °C at the end of 60 min.

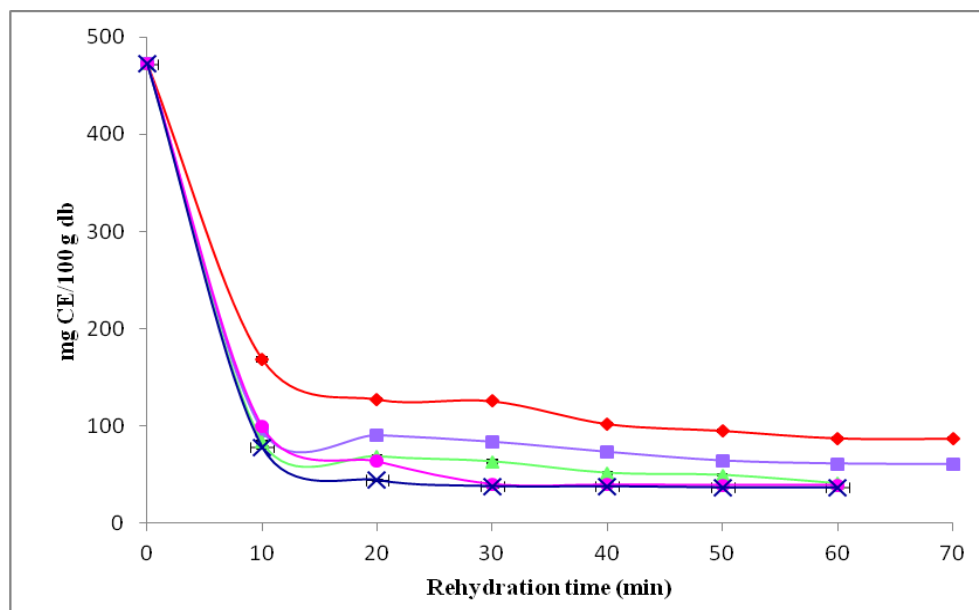


Fig. 6.14 Effect of rehydration temperatures on the total tannin content of *H. elongata* (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C)
Each value is presented as mean \pm SD (n = 6).

As every thermal process can be detrimental to the integrity of plant tissue, particularly cellular membranes temperature, increases above the optimum could lead to damage in the matrix leading to variations in its response to the process being applied. Accordingly, increasing rehydration temperature causes a deterioration of texture due to the damage caused during thermal dehydration and promotes significant loss of mechanical resistance in the samples. This excess softening of tissues alters mass transfer ability of the system. During rehydration, sugars solubilise and molecules become more mobile, which increase solid loss throughout the processing time. If the temperature is raised these effects are intensified (Maldonado *et al.*, 2010).

The free radical scavenging activity of *H. elongata* rehydrated for 60 min was determined by the DPPH radical scavenging assay tested at a concentration of 50 µg/ml extract (Fig. 6.15). Initial dried *H. elongata* extract had a DPPH radical scavenging activity of 75.7%. Rehydration at all temperatures resulted in an increase in DPPH scavenging activity of the extract. The activity increased by 11.5, 18.0, 25.3, 27.5 and 18.2% at 20, 40, 60, 80 and 100 °C, respectively. These results show that there is a temperature dependence in the case of antioxidant activity. It has been reported that blanching of vegetables similar to that in the present rehydration study, positively affects the radical scavenging activity, and Rossi *et al.* (2003) showed that blanching strongly lowered the signal amplitude of DMPO-OH radical adduct in comparison with an unblanched sample using electron paramagnetic resonance (EPR) spectroscopy, hence increasing its activity.

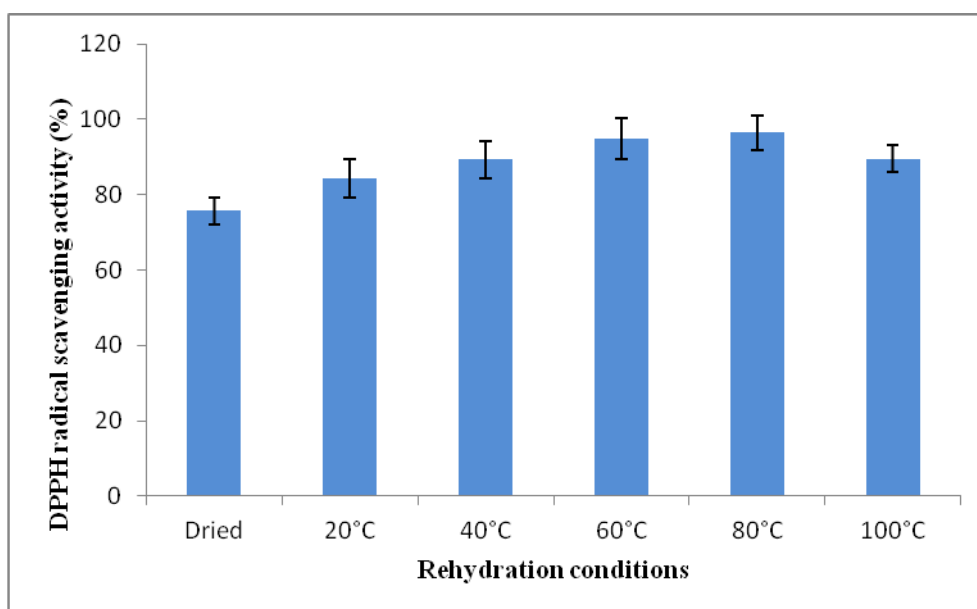


Fig. 6.15 Effect of rehydration temperatures on the DPPH radical scavenging activity of dried *H. elongata*

Each value is presented as mean \pm SD (n = 6).

6.3.8 Effect of dehydration and rehydration on the texture and SEM imaging of *H. elongata*

The texture of fresh and dried *H. elongata* is quite tough and needs some processing to soften. In the present work, it was found that a combination of drying before rehydrating in hot water not only gives seaweed an edible texture, but the dehydration pre-treatment reduces this processing time. Thermal processing brings about changes in the microstructure and hence the texture. The initial texture of fresh seaweed was 45.90 ± 1.88 N/mm which increased steadily within the initial 5 to 6 hours of dehydration. This corresponded to the seaweed becoming more stretchy, after which it became hard and brittle. Due to this variation; the first 5 (40 °C) to 6 (25, 30 and 35 °C) hours of the drying procedure were modelled until texture was at its maximum.

With regard to the effect of temperature on textural parameters during dehydration and rehydration, several models based on expressions for reaction rate are available in the literature and generally zero and first-order models are applied (Nisha *et al.*, 2006). Accurate knowledge of the kinetic parameters, degradation rate constant and activation energy are essential to predict quantitatively the quality changes that occur during thermal processing (Nisha *et al.*, 2010). In the present study, a first-order model was successfully employed for modelling the effect of dehydration kinetics on texture and a zero-order model was employed for the rehydration kinetics of texture of *H. elongata*. Similar models have been applied to model the texture in various foods such as sesame seeds and potato (Nisha *et al.*, 2006; Kahyaoglu and Kaya, 2006).

In order to describe the dehydrated texture kinetics of seaweed a first-order model was employed (Eq. 3.19) and the model provided a good agreement with the experimental data. The correlation coefficients (R^2 values) ranged from 0.9014 to 0.991 for the different temperatures. Thus, the first-order model was assumed to adequately describe texture changes. Fig. 6.16 shows the predicted and experimental points obtained for the four temperatures for the first order model. The examination of the parameters obtained from the model (Table 6.8) showed that the kinetic rate constant parameter ' k ' increased as the temperatures increased. The ' C ' parameter of the model also increased at the higher dehydration temperatures. To prove the dependence of parameter k on the drying temperature (Vega *et al.*, 2007), the Arrhenius equation was applied, graphically representing $\ln k$ versus $1/T$ (Simal *et al.*, 2005). A straight line was obtained with a regression coefficient (R^2) of 0.8781 (Fig. 6.18a), and an activation energy of 0.0058 J/mol.

The texture kinetics of rehydrated *H. elongata* was described using a zero-order model which provided a good agreement with the experimental data. R^2 values ranged from 0.8079 to 0.9277. The initial texture of the dried seaweed (dried for 24 h) applied in the rehydration study was 85 N/mm and modelling of texture kinetics was investigated over the entire 70 min rehydration time. During cooking, the texture softens as a result of different chemical and physical changes take place. The texture change varied in the range of 43.0 to 25.6 N/mm upon rehydration with the greatest reduction seen at the highest temperature of 100 °C. Fig. 6.17 shows the predicted and experimental points obtained for the five rehydration temperatures studied for the zero-order model. The kinetic rate constant parameter k increased as the temperatures increased (Table 6.9). The texture of the seaweed (Fig. 6.17) softened

significantly over the rehydration process for each of the temperatures studied ($P < 0.05$).

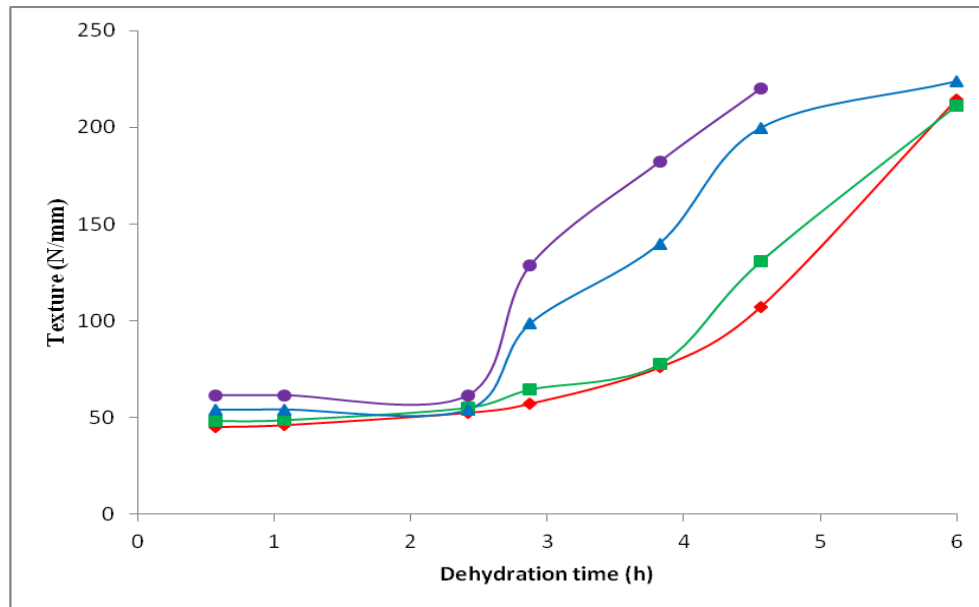


Fig. 6.16 Experimental and predicted texture of *H. elongata* during dehydration at four temperatures (♦: 25 °C; ■: 30 °C; ▲: 35 °C; ●: 40 °C) Points represent the experimental values and lines represent the predicted values. Each value is presented as mean \pm SD (n = 6).

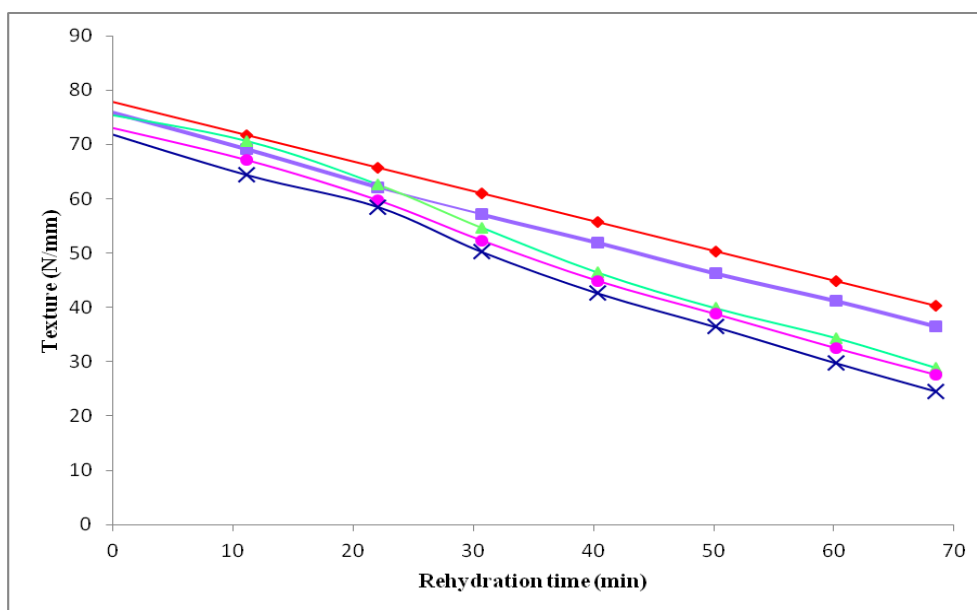


Fig. 6.17 Experimental and predicted texture of *H. elongata* during rehydration at five temperatures (♦: 20 °C; ■: 40 °C; ▲: 60 °C; ●: 80 °C; × 100 °C)
Points represent the experimental values and lines represent the predicted values.

Each value is presented as mean \pm SD (n = 6).

The force required to break the initial dried seaweed was 85.0 N/mm. The texture change varied within the range of 43.0 to 25.6 N/mm upon rehydration with the greatest reduction seen at the highest temperature of 100 °C. The trend of reduction in phytochemicals varied in accordance with the reduction in texture for each of the rehydration temperatures. Besides the water uptake, during the rehydration process, there is also a soluble solid loss, which could produce significant losses of vitamins, sugars, amino acids and minerals (García-Pascual *et al.*, 2006).

Table 6.8 Results of fitting dehydration kinetics (values in curved brackets are the standard error and values in square brackets are 95% confidence intervals)

Model	Parameter	25 °C	30 °C	35 °C	40 °C
First Order	<i>C</i>	43.61 (±5.96) [29.01-58.21]	47.98 (±2.46) [41.96- 54.00]	54.19 (±7.59) [34.67- 73.71]	61.45 (±9.73) [36.46- 86.46]
	<i>k</i>	0.85 (±0.006) [0.84-0.87]	0.91 (±0.01) [0.88- 0.94]	1.03 (±0.02) [0.98-1.09]	1.02 (±0.29) [0.94-1.09]

* *C* and *k* are the model parameters

Table 6.9 Results of fitting rehydration kinetics (values in curved brackets are the standard error and values in square brackets are 95% confidence intervals)

Model	Parameter	20 °C	40 °C	60 °C	80 °C	100 °C
Zero Order	<i>C</i>	77.82 (±2.61) [71.42- 84.22]	75.27 (±3.69) [66.24- 84.32]	72.04 (±5.01) [60.19- 83.88]	69.25 (±5.18) [56.99- 81.51]	67.78 (±5.17) [55.53- 80.02]
	<i>k</i>	0.55(±0.06) [0.39-0.70]	0.57 (±0.09) [0.35- 0.79]	0.71 (±0.13) [0.40- 1.02]	0.74 (±0.13) [0.44- 1.04]	0.74 (±0.13) [0.43- 1.05]

* *C* and *k* are the model parameters

The C parameter of the model decreased with the higher dehydration temperatures. The Arrhenius equation was applied and gave a straight line with a regression coefficient (R^2) of 0.8748 (Fig. 6.18b), and an activation energy of 0.0157 J/mol. The low activation energy of seaweeds clearly indicates that the texture of seaweeds is very tough and can degrade easily upon the application of even small amount of heat. Estimated activation energies for texture of food products in the literature are extremely varied. Vu *et al.* (2004) reported the activation energy of carrot texture to be 117.6 kJ/mol whereas Kahyaoglu and Kaya (2006) found the activation energy of sesame seeds to be 6.9 kJ/mol.

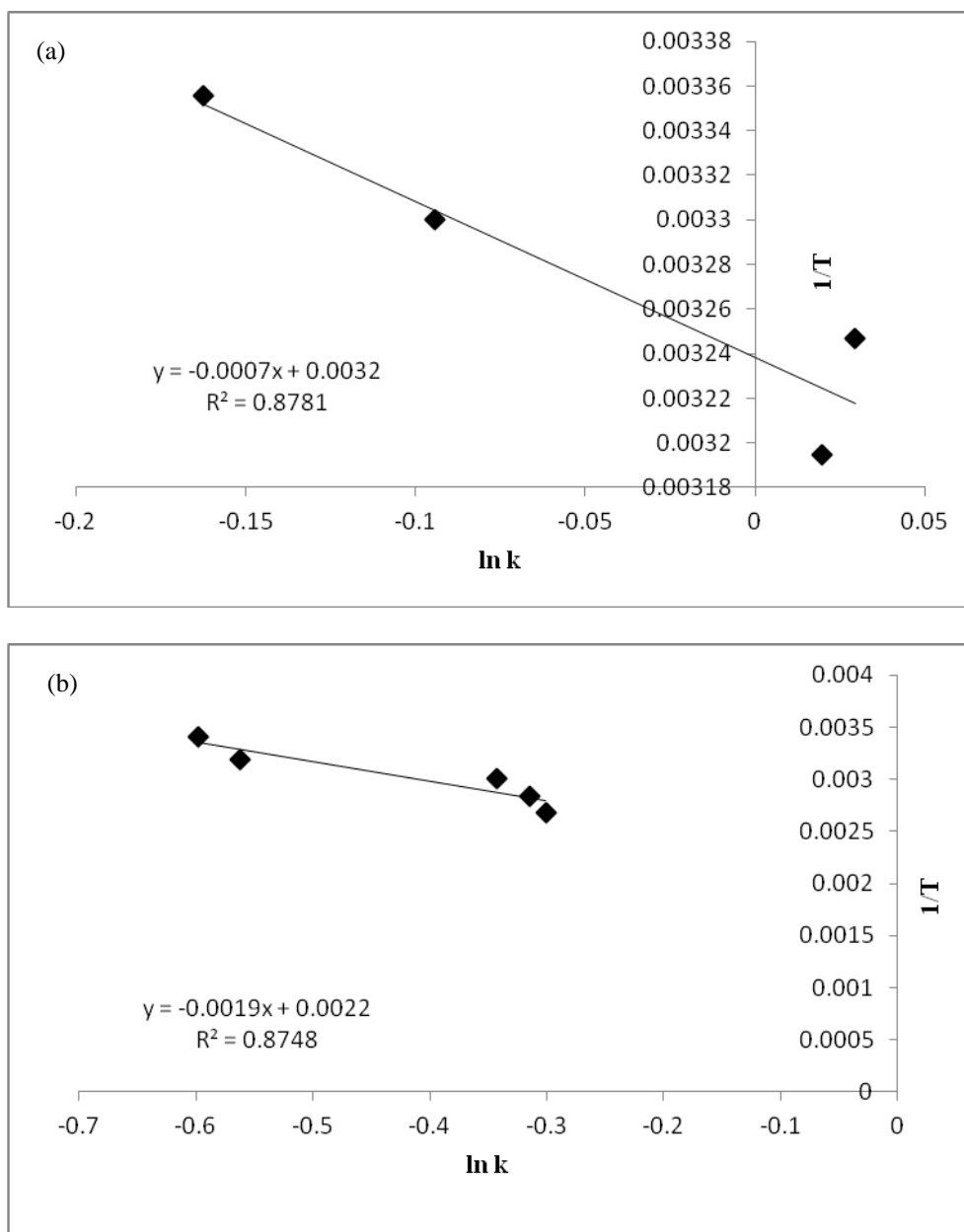


Fig. 6.18 Graphical representation of the influence of temperature on parameter (k) of the exponential model during dehydration (a) and rehydration (b). $1/T$ (kJ/mol).

Image analysis was carried out on the fresh, dried and rehydrated seaweed tissues using scanning electron microscopy (SEM) analysis as in Fig. 6.19. An image of fresh *H. elongata* can be observed in Fig. 6.19a; the structure became flattened, slightly collapsed and shrinkage in the seaweed fronds was observed upon drying (Fig. 6.19b). The number of open structures and pores became considerably lesser

upon drying. Seaweed rehydrated at 100 °C for 40 min (equilibrium moisture content) can be seen in Fig. 6.19c. In this case, the structure of the rehydrated seaweed has become more swollen in comparison to fresh and dried *H. elongata* which could be attributed to the significant texture change upon rehydration of the seaweed at such high temperatures. García-Segovia *et al.* (2011) reported similar structural findings in rehydrated mushroom.

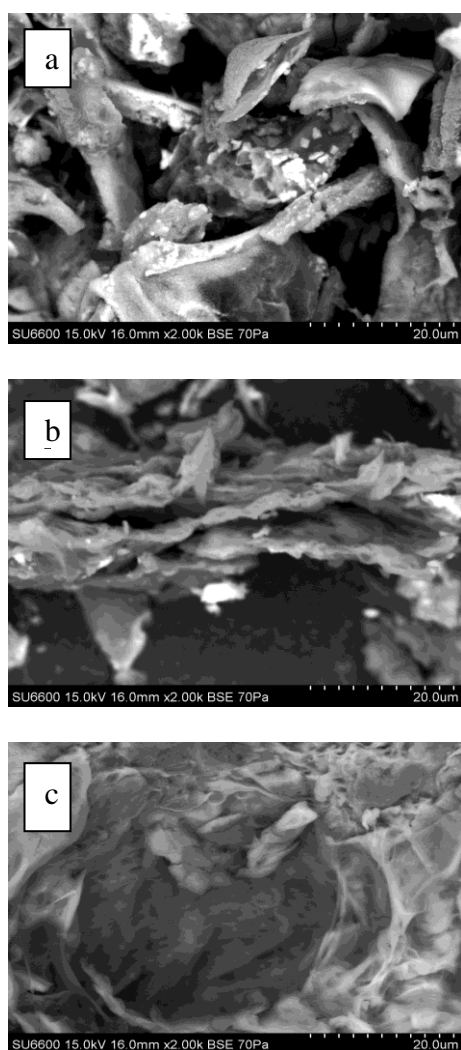


Fig. 6.19 SEM of *H. elongata* tissue (a) fresh, (b) dried at 40 °C, 24 hours and (c) rehydrated at 100 °C for 40 min

6.3.9 Effect of dehydration and rehydration on the colour of *H. elongata*

Colour has been recognised as an important element in food choice, often reflecting our expectations with regard to flavours (Hutchings, 2003; Lavin and Lawless, 1998; Leon *et al.*, 1999). During the dehydration of *H. elongata* the colour darkens from brown to almost black and once the seaweed is rehydrated in hot water, it becomes a bright green colour. Colour characteristics are important as quality attributes for dehydrated and rehydrated foods. For most vegetables, the colour should closely resemble the colour characteristics of the fresh food material to increase acceptability. Rehydration of dried *H. elongata* in hot water leads to a colour change from brown to green which could be considered beneficial since the green colour of vegetables is one of the major sensory characteristics in determining the final quality of thermally processed foods. In this study, the change of colour was determined by the CIE $L^*a^*b^*$ system frequently used as a versatile and reliable method to assess the colour of fruit and vegetables (Moireira *et al.*, 2008; Vega-Gálvez *et al.*, 2009).

Table 6.10 shows the average values of the chromatic coordinates L^* , a^* and b^* , for the fresh, dehydrated and rehydrated samples of *H. elongata* at the end of the drying (24 h) and rehydration (60 min) period. Coordinate L^* decreases as the drying temperatures are increased and the samples change from dark brown to black in colour and then upon rehydration L values increase with higher temperatures as seaweeds turn green. The L^* value of the dried seaweed was significantly different from fresh ($P < 0.05$). This indicates that the fresh and dried samples presented a darker colour compared to the rehydrated samples. Dehydration led to an increase in a^* (greenness-redness) values with increasing temperature up to 84.1% at 40 °C, as compared to fresh seaweed ($P < 0.05$). Dried seaweed (40 °C, 24 h) increased

considerably in coordinate a^* values during rehydration from 0.31 ± 0.09 to 4.97 ± 0.16 at 100 °C. In comparison to fresh seaweed, the a^* values of the rehydrated dried seaweed increased up to 154.8% (100 °C, 60 min).

Table 6.10 Colour of *H. elongata* before and after dehydration and rehydration

Processing condition	L*	a*	b*	ΔE
Fresh seaweed	13.42 ± 1.09	1.95 ± 0.23	3.77 ± 0.45	-
Dehydration				
25 °C	11.48 ± 0.98	0.64 ± 0.15	1.95 ± 0.21	2.96 ± 0.23
30 °C	10.47 ± 1.11	0.43 ± 0.12	0.23 ± 0.06	4.85 ± 0.56
35 °C	10.23 ± 1.09	0.32 ± 0.18	0.19 ± 0.08	5.07 ± 0.45
40 °C	9.62 ± 1.05	0.31 ± 0.09	0.18 ± 0.07	5.21 ± 0.21
Rehydration				
20 °C	14.27 ± 1.23	0.91 ± 0.10	4.49 ± 1.05	1.55 ± 0.33
40 °C	16.42 ± 1.09	1.09 ± 0.08	7.85 ± 1.21	5.24 ± 0.24
60 °C	16.80 ± 1.14	1.19 ± 0.09	8.03 ± 0.98	5.59 ± 0.32
80 °C	17.70 ± 1.56	2.62 ± 0.12	11.05 ± 1.11	8.61 ± 0.89
100 °C	17.92 ± 1.23	4.97 ± 0.16	12.10 ± 1.21	10.09 ± 1.02
Each value is presented as mean \pm SD (n = 6).				

Coordinate b^* (blueness – yellowness) showed a decrease in its value upon dehydration in the range of 48.2 to 95.2% from 25 to 40 °C. Jokić *et al.* (2009) also found that dehydration temperature was an important factor in the drying of asparagus where increasing the drying temperature caused a greater colour change resulting in a darker colour. The b^* values then significantly increased upon rehydration from 0.18 ± 0.07 to 12.10 ± 1.21 in samples rehydrated at 100 °C ($P < 0.05$). Total colour difference (ΔE) is a function of the three CIE L^* a^* b^*

coordinates (Eq. 3.3) and is extensively used to characterise the variation in colour in foods during processing. Changes from 2.96 ± 0.23 to 5.21 ± 0.21 in ΔE values were estimated for dehydration from 25 to 40 °C. Similar results were obtained by Vega-Gálvez *et al.* (2009) and Guiné and Barroca (2011), where large ΔE values were estimated at higher drying temperatures due to the effect of high temperatures on heat-sensitive components like carbohydrates and proteins. In the case of rehydrated samples, the ΔE values also increased with increasing temperature in the range of 1.55 ± 0.33 to 10.09 ± 1.02 from 20 to 100 °C, which signifies that there was a greater colour change in samples as temperature increased ($P < 0.05$). Similar results were observed by Moreira *et al.* (2008).

6.4 Conclusion

The results of this chapter showed that the drying kinetics of seaweeds can be accurately predicted using the empirical models of Newton, Logarithmic or Henderson–Pabis models. The moisture transfer can be described by diffusion and the temperature dependence of the effective moisture diffusivities was shown to follow an Arrhenius relationship. Dehydration reduced the phytochemical constituents in the seaweed. A reduction of 29% in the TPC and 30% in the TFC was seen when *H. elongata* was dried at 40 °C. However, an important increase of 41% in the TPC was nonetheless observed when the seaweed was dried up to 50% moisture content. Seaweed dried at 40 °C for 24 h had a 12.3% increase in TTC and reduction in DPPH scavenging was minimal under the same conditions with only a 4.5% loss of activity. This would mean that the semi-dried form of seaweeds which is even more nutritious than the raw state could be used for the development of health promoting seaweed based products. However, the results also showed that

processing of *H. elongata* by drying resulted in a substantial reduction of the phytochemicals which leads to the fact that new research into protecting antioxidant properties of seaweeds upon processing would be needed. The rehydration kinetics of *H. elongata* can be accurately predicted using the empirical models of Weibull, Peleg's, first-order and exponential association. The temperature dependence of the rehydration procedure was shown to follow an Arrhenius relationship. Overall, restoration of the product to its original moisture content was achieved however there were significant losses in phytochemical content during the rehydration process.

Moisture equilibrium was achieved most quickly at 100 °C after 40 min. Under these rehydration conditions, losses of up to 93% were seen in the phytochemicals, as compared to dried seaweed. Since dried seaweeds are generally rehydrated prior to consumption, the present study clearly shows that the process of drying and subsequent rehydration can significantly reduce the phytochemicals present in seaweeds. Thereby it is important to find out novel methods of rehydration which could prevent the destruction of these important properties of seaweeds. Rehydration or cooking of food materials improves digestibility, palatability, gives microbial safety and can change the colour and texture of the product. Colour and texture were also significantly changed during the dehydration and rehydration procedures and modelling of texture kinetics were successfully fitted to zero and first-order models. The present study therefore demonstrates that the dehydration and rehydration process needs to be controlled in order to minimise phytochemical losses while at the same time processing the product to an edible texture.

CHAPTER 7

OPTIMISATION OF HYDROTHERMAL PROCESS CONDITIONS (REHYDRATION) OF SEMI-DRIED *H.* *ELONGATA*

Optimisation of phytochemical content, colour and texture of semi-dried H. elongata during hydrothermal processing (rehydration) using Response Surface Methodology (RSM)

*The results from this chapter were published as a peer-reviewed article in the
Botanica Marina Journal*

Summary

Response surface methodology was applied to investigate the effect of time and temperature of hydrothermal processing (rehydration) on the phytochemical content, texture and colour of semi-dried *H. elongata* (dried for 2 hours at 40 °C). A central composite design was employed with a rehydration time of 10 – 30 min and temperature of 60 – 90 °C. Predicted models were found to be significant ($P < 0.05$) for total phenolic content, DPPH radical scavenging activity, total flavonoids, total condensed tannins, texture and colour. Predicted values for each of the responses were in good agreement with the experimental values. Processing time had most significant effect on phytochemical constituents of *H. elongata*. An acceptable edible texture and colour of seaweed was also achieved during the rehydration procedure. Thus, central composite design and response surface methodology can be used to model phytochemical content, texture and colour of *H. elongata*. Multiple response optimisation demonstrated that phytochemical content of *H. elongata* may be maximised by rehydration in hot water at 80.5 °C for 20.4 min.

7.1 Introduction

Marine algae have been used as foods in Asia in the preparation of salads, soups and also as low-calorie foods (Jiménez-Escrig and Sanchez-Muniz, 2000) and in Western countries, mainly as a source of polysaccharides (agar, alginates and carrageenans). As in other photosynthetic organisms, seaweeds contain various kinds of inorganic and organic substances that may benefit human health. Seaweeds contain high levels of minerals, vitamin, essential amino acids, indigestible carbohydrates and dietary fibre (Jiménez-Escrig and Goni, 1999).

Several seaweeds are perishable in their fresh state and deteriorate within a few days after harvest. The traditional way to preserve these plant products is by sun drying in order to conserve their desirable qualities, reduce storage volume and to extend shelf life (Lim and Murtijaya, 2007). In Ireland the dehydration of seaweed is carried out under atmospheric conditions outdoors and therefore due to weather changes the drying conditions will not always be similar or controlled. Many dried seaweeds are rehydrated and cooked by immersion in boiling water. Processing and preparation of vegetables, especially thermal treatment, which are applied prior to consumption may affect phytochemical content. Phenolic compounds are particularly sensitive to heat, whereby boiling of vegetables for few minutes may cause a significant loss of phenolic content leaching into water (Amin *et al.*, 2006). Since seaweeds are often consumed after processing such as dehydration and rehydration, it is important to investigate the effect of rehydration time and temperature on their phytochemical content in order to develop an optimised processed product.

The ‘one-at-a-time-approach’ can be used to optimise the processing time and temperature of the rehydration procedure. However, this method is extremely time consuming and disregards the complex interactions among various physicochemical parameters. In order to optimise the processing parameters, response surface methodology (RSM) was applied. RSM is a suite of mathematical and statistical techniques used to search for optimum conditions of factors for desirable responses, and evaluating the relative significance of several treatment factors even in the presence of complex interactions. The design leads to the generation of contour plots by linear or quadratic effects of the key variables and a model equation is derived

that fits the experimental data to calculate the optimal response of the system (Liyana-Parthirana and Shahidi, 2005; Zhang *et al.*, 2007 and Prasad *et al.*, 2011).

The main aims of this chapter were as follows:

1. To apply response surface methodology to optimise the time and temperature of the rehydration conditions for seaweeds that had previously been semi-dried (40 °C, 2 hours).
2. To investigate the effects of rehydration time and temperature on the phytochemical content of *H. elongata*.
3. To assess the effect of rehydration on the colour and texture of *H. elongata*.
4. To develop a model equation to predict and determine optimum rehydration conditions for semi-dried seaweed.

7.2 Materials and methods

7.2.1 Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in January 2010 for the dehydration study and in August 2010 for the rehydration study and stored at 4 °C until further analysis.

7.2.2 Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before processing.

7.2.3 Drying pre-treatment

Dehydration was carried out as described in section 3.12.1 at 40 °C for 2 hours.

7.2.4 Hydrothermal processing (Rehydration)

Rehydration of the semi-dried seaweed samples was carried out as described in section 3.12.2. The hydrothermal processing treatments applied are presented in Table 7.1. After processing, the cooked seaweeds were drained in a wire mesh strainer and placed on ice to cool before the extraction procedure.

7.2.5 Experimental design

A central composite design with two factors was utilised to investigate the effect of factors (rehydration time and temperature) on phytochemical constituents of *H. elongata*. The central composite design was applied using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA, USA). The total number of experiments generated from the software with two factors were 10 ($= 2^k + 2k + 2$), where k is the number of factors. Eight experiments were augmented with two duplicates at the centre points. The variable combinations in experimental runs are shown in Tables 7.1 and 7.2 below.

Table 7.1 Design matrix and variable combinations in experimental runs

Experiment No.	Rehydration time (min)	Rehydration temperature (°C)
1	20.00	75.00
2	20.00	96.21
3	20.00	53.78
4	5.85	75.00
5	20.00	75.00
6	30.00	60.00
7	10.00	90.00
8	10.00	60.00
9	34.14	75.00
10	30.00	90.00

The independent variables of hydrothermal processing were rehydration time (10 - 30 min) and temperature (60 – 90 °C). Experimental data from the central composite design was analysed and fitted to a polynomial regression model as seen below:

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_{11} \chi_1^2 + \beta_{22} \chi_2^2 + \beta_{12} \chi_1 \chi_2 \quad \text{Eq. 7.1}$$

Where; Y is response calculated by the model; β_0 is a constant and β_i , β_{ii} and β_{ij} are linear, squared and interaction coefficients, respectively.

The adequacy of the model was evaluated by the lack of fit, coefficient of determination (R^2) and the Fisher's test value (F -value) obtained from the analysis of variance (ANOVA) generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level ($\alpha = 0.05$). Three-

dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables). The independent variables selected are presented in Table 7.2.

Table 7.2 Level of codes for independent variables used in the central composite design

Independent variables	Symbol	-2	-1	0	+1	+2
Rehydration time (min)	X ₁	5.85	10	20	30	34.14
Rehydration temperature (°C)	X ₂	53.78	60	75	90	96.21

A multi-response analysis of the response surface design was performed using the desirability approach to optimise blanching time and temperature. The desirability function is an approach for solving the problem of optimisation of several responses and is applied when various responses have to be considered at the same time and it is necessary to find optimal compromises between the total numbers of responses taken into account. This methodology is based on first constructing a desirability function for each individual response, and then it is possible to obtain the overall desirability.

7.2.6 Texture evaluation

At specified experimental time seaweed samples (original 5 g fw) were removed to undergo instrumental texture analysis. Shear tests were performed as described in section 3.9.

7.2.7 Colour measurement

At specified experimental time seaweed samples (original 5 g fw) were removed to undergo colour analysis using a colourimetry (CIE Lab ColourQuest XE, Hunter Associates, Reston, VA, USA) as described in section 3.10.

7.2.8 Extraction of phytochemicals

Seaweed samples (original 5 g fw) were powdered in liquid nitrogen using a mortar and pestle, then extracted as described in section 3.2.

7.2.9 Total phenolic content

The total phenolic content of seaweed samples was measured using the Folin-Ciocalteu method (Taga *et al.*, 1984) as described in section 3.4. The total phenolic contents of the whole seaweeds were expressed as mg gallic acid equivalent per 100 gram fresh weight (mg GAE/100 g fw).

7.2.10 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as described in section 3.7. Results were expressed as % reduction in DPPH radical scavenging activity.

7.2.11 Total flavonoid content

Total flavonoid content was determined as described in section 3.5. Results were expressed as mg quercetin equivalents (QE)/100 gram fresh weight (mg QE/100 g fw).

7.2.12 Total condensed tannin content

Total condensed tannin content was determined as described in section 3.6. Results were expressed as mg catechin equivalents (CE)/100 gram fresh weight (mg CE/100 g fw).

7.2.13 Statistical analysis

All experiments were carried out in triplicate and replicated at least twice. Data from the central composite design were subjected to a second-order multiple regression analysis using least-squares regression to obtain the parameters estimated for the mathematical model. The regression analysis and analysis of variance (ANOVA) were performed with the STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). Differences were considered statistically significant when $P < 0.05$.

7.3 Results and Discussion

7.3.1 Statistical analysis of results obtained by experimental design

Processing and preparation of vegetables, (especially thermal treatment), applied prior to consumption may affect the phytochemical level of the food. Heat applications such as boiling are common practices in the processing of vegetable

products in order to render them palatable and microbiologically safe. Since dried seaweed would need to undergo some rehydration and heat treatment prior to consumption, it was relevant to assess the effects of hydrothermal treatment or rehydration in hot water, on the stability of seaweed antioxidant properties. Seaweeds are perishable in their fresh state and deteriorate within a few days of harvest and are most commonly dried outdoors under atmospheric conditions as a means of preservation.

In chapter 6, the effect of a range of dehydration temperatures on the drying kinetics and phytochemical constituents of *H. elongata* was investigated and results showed that drying at 40 °C for 2 hours increased the phytochemical content as compared to fresh seaweeds (Gupta *et al.*, 2011). Therefore this optimised semi-dried seaweed was utilised in the present study in order to develop a rehydrated seaweed with optimal phytochemical levels.

Phenolic compounds are sensitive to heat, so immersion of vegetables in hot water for few minutes may cause a significant loss of phenolic content which can leach into boiling water (Amin *et al.*, 2006). Therefore, in the present chapter, time and temperature of rehydration were chosen as factors for the RSM study as they have major effects on phytochemical levels. Effects of independent variables for each of the response variables are presented in Table 7.3.

Table 7.3 Two-way ANOVA for the effects of independent variables on the response of variables: total phenolic content, DPPH, total flavonoids, total condensed tannins, texture and colour of *H. elongata*

Source	Total phenolic content	DPPH	Total flavonoids	Total condensed tannins	Texture	Colour
	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -value
X ₁	198.68	0.0001	77.22	0.0009	85.26	0.0008
X ₂	25.55	0.0072	1.24	0.3273	0.64	0.4675
X ₁ *X ₁	107.81	0.0005	52.36	0.0019	2.00	0.2303
X ₁ *X ₂	68.71	0.0012	25.07	0.0075	4.91	0.0911
X ₂ *X ₂	86.06	0.0008	123.94	0.0004	21.78	0.0095

***R*² values: 0.9908 (total phenolic content), 0.9832 (DPPH), 0.9658 (total flavonoids), 0.9528 (total condensed tannins), 0.9174 (texture) and 0.9407 (colour)**

Statistical analysis indicated that the mathematical models proposed were adequate with no significant lack of fit and had high R^2 values for all of the responses. The models for each of the responses were analysed separately before an overall optimum processing condition of the hydrothermal processing procedure was determined. Predicted and experimental values for each of the responses are presented in Table 7.4. Response surface plots were generated to illustrate the effects of blanching time and temperature on each of the responses (Fig. 7.1 – 7.6).

Table 7.4 Predicted (Pred.) and experimental (Exp.) values of total phenolic content, DPPH radical scavenging activity, total flavonoids, total condensed tannins, texture and colour of *H. elongata*

Experiment No.	Total phenolic content (mg GAE/100 g fw)		DPPH (%)		Total flavonoids (mg QE/100 g fw)		Total condensed tannins (mg CE/100 g fw)		Texture (N/mm)		Colour (ΔE)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	64.19	64.24	99.56	99.13	11.69	12.00	11.65	11.90	31.93	31.80	10.37	10.07
2	47.63	48.24	91.21	91.27	8.67	8.76	8.82	8.30	34.70	33.31	13.67	13.68
3	54.13	55.45	92.22	92.07	8.66	9.31	10.63	11.86	31.41	32.28	9.92	9.30
4	59.27	60.43	90.54	91.14	13.61	14.23	17.78	17.84	40.49	40.86	10.36	10.37
5	64.28	64.24	98.71	99.13	12.31	12.00	12.15	11.90	31.68	31.80	9.78	10.07
6	53.55	52.46	96.72	97.28	9.18	8.79	11.80	10.61	33.09	33.00	10.34	11.09
7	62.43	61.59	92.76	92.26	13.19	12.84	13.34	13.82	36.39	37.01	12.99	12.85
8	56.21	54.86	89.59	89.24	12.47	11.72	16.56	15.79	38.59	37.60	8.31	8.62
9	39.53	40.31	98.11	97.43	7.85	7.97	9.10	9.75	37.10	36.21	12.88	12.26
10	36.10	35.52	92.72	93.13	6.90	6.91	7.48	7.54	33.53	35.04	12.75	13.05

Values are presented as mean \pm SD (n = 6)

7.3.2 Effects of process variables on total phenolic content

Time and temperature combinations used for the processing of vegetables are crucial in optimising the concentration of bioactive compounds. RSM was applied to obtain conditions which would result in minimal loss of phytochemicals into water during rehydration. The 10 experiments proposed by the response surface methodology with two factors and five levels (Table 7.2), including two replicates at the centre point were used for fitting a second-order response surface model. The two centre point runs provided a measure of process stability and inherent variability. Experimental results for total phenolic content (TPC) were fitted to a full quadratic second order polynomial equation and the model obtained for TPC of hydrothermally processed *H. elongata* was:

$$Z = -150.541 + 5.02044*X_1 + 4.74852*X_2 - 0.0693325*X_1^2 - 0.0394485*X_1*X_2 - 0.0275309*X_2^2$$

Eq. 7.2

(See Table 7.2 for definitions of X_1 and X_2). In order to determine the significance of the model, an ANOVA was performed on the data. For each experimental factor, the variance was partitioned into linear, quadratic and interaction components, in order to assess the adequacy of the second order polynomial function and the relative importance or significance of the terms. The coefficients of the model are estimated with multiple regression analysis. The quality of fitting of the second order equation was expressed by the coefficient of regression R^2 . The R^2 values provided a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The goodness of fit of the model was examined by an F -test. The F -test is a statistically valid measure of how well the

factors describe the variation in the data about its mean. The greater the deviation of the F -value from unity, the more certain it is that the factors explain adequately the variation in the data around its mean, and the estimated factor effects are real. The F -values for each of the factors of TPC were very high, up to 198.68 (Time X_1) indicating that these factors are highly significant (Table 3). The model explained 99.08% (R^2 of 0.9908) of the variation in TPC.

The P -values were used to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the P -value, the larger the significance of the corresponding coefficient. Corresponding P -values indicate that, among the test variables and their interactions, X_1 (rehydration time), X_2 (rehydration temperature), $X_1 \times X_1$ (time \times time), $X_1 \times X_2$ (time \times temperature) and $X_2 \times X_2$ (temperature \times temperature) were significant model terms with P -values < 0.05 . Therefore it is important to find the optimum rehydration time and temperature at which the TPC loss is minimised while at the same time making the product edible.

The polynomial response models were expressed as three-dimensional (3D) surface plots to better visualize the relationship between the rehydration time and temperature as independent variables and phytochemical properties as response variables. The fitted response surface plots were generated by statistically significant models using Statgraphics software to illustrate the interaction of the parameters required for optimum responses. The response plot (Fig. 7.1) shows that rehydration time and temperature both had significant effects on the TPC, with significant interaction between the factors. The shape of the contour plots (circular or elliptical)

indicates whether the mutual interactions between variables are significant or not. A circular contour plot indicates that the interactions are negligible. An elliptical contour plot indicates that the interactions between related variables are significant (Muralidhar *et al.*, 2001). The response surface plot showed that increasing blanching time and temperature resulted in decreases in the TPC.

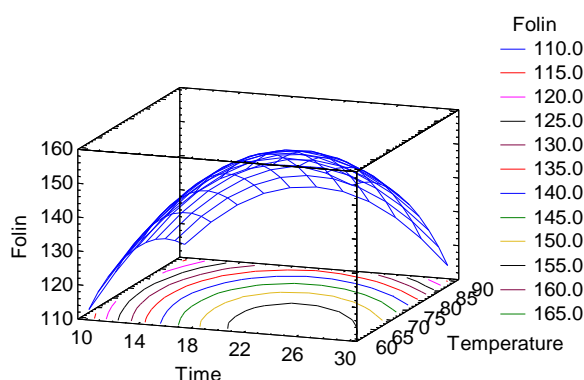


Fig. 7.1 Response surface plot showing effects of rehydration time (min) and temperature (°C) on the total phenolic content (GAE/100 g fw) of *H. elongata*

Processing dried or semi dried seaweed in hot water for a specific time allows time for both rehydration and for the seaweed to become edible as heat will reduce the toughness. Phenolic compounds are sensitive to heat and boiling of vegetables can cause a significant loss of phenolic content which can leach into water which was the case of Xu and Chang (2008) who found a 40 – 50% loss in the TPC of legumes. Bunea *et al.* (2008) also reported a 50% loss of TPC in spinach hydrothermally processed for 10 min. In the present study, a reduction in TPC from 142.6 to 64.2 mg GAE/100 g fw was observed, when the seaweed was rehydrated at 75 °C for 20 min, most likely due to leaching of phytochemicals into the water.

7.3.3 Effects of process variables on DPPH radical scavenging activity

The model obtained for the DPPH radical scavenging activity of hydrothermally processed *H. elongata* extract was:

$$Z = -24.7231 + 2.08795 \cdot X_1 + 2.7056 \cdot X_2 - 0.0242313 \cdot X_1^2 - 0.01195 \cdot X_1 \cdot X_2 - 0.0165694 \cdot X_2^2$$

Eq. 7.3

There were significant ($P < 0.05$) influences of the linear factors of X_1 (rehydration time), and all interactions $X_1 \cdot X_1$ (time \times time), $X_1 \cdot X_2$ (time \times temperature) and $X_2 \cdot X_2$ (temperature \times temperature) on the DPPH radical scavenging activity. X_2 (rehydration temperature) had no significant linear effect on the model ($P > 0.05$) (Table 7.3). The fit of the model was further confirmed by a high coefficient of determination, 0.983. The response surface plots generated showed slight increases in DPPH radical scavenging activity as rehydration time increased (Fig. 7.2).

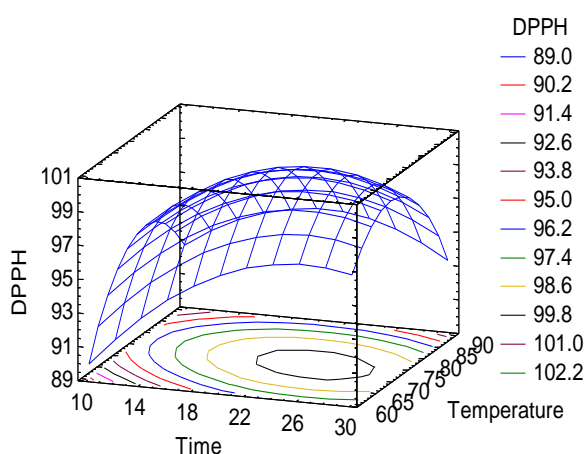


Fig. 7.2 Response surface plot showing effects of rehydration time (min) and temperature (°C) on DPPH radical scavenging activity (%) of *H. elongata*

The DPPH scavenging results of the seaweeds from the present study are more effective than some outlined in literature for other vegetables. Thermal processing often increases the DPPH radical scavenging activity per unit mass of extract in vegetables. Turkman *et al.* (2005) found that boiled greenbeans and peas had 70.8% and 17.9% scavenging activity at 6 mg/ml extract concentration, respectively, *H. elongata* rehydrated for 20 min at 75 °C showed 99.5% scavenging activity at a much lower concentration of extract (50 µg/ml). Possible reasons could be that the compounds in the *H. elongata* extract were more potent than those of greenbeans so possessed a higher activity at lower extract concentrations.

7.3.4 Effects of process variables on total flavonoid content

Flavonoids are another important phytochemical found in vegetables that can be lost during processing. It is important to maintain the levels of flavonoids from a nutritional and functional point of view as they are known to possess antioxidant properties. The model obtained for total flavonoid content of rehydrated *H. elongata* was:

$$Z = -28.9533 + 0.33379 \cdot X_1 + 1.07504 \cdot X_2 - 0.00448804 \cdot X_1^2 - 0.00501146 \cdot X_1 \cdot X_2 - 0.00658426 \cdot X_2^2 \quad \text{Eq. 7.4}$$

The linear factor of X_1 (rehydration time) had a significant influence on the model ($P < 0.05$) while X_2 (rehydration temperature) and the quadratic interaction factors, $X_1 \cdot X_1$ (time \times time), $X_1 \cdot X_2$ (time \times temperature) did not ($P > 0.05$). The quadratic effect of temperature, $X_2 \cdot X_2$ was significant ($P < 0.05$). The fit of the model was further confirmed by a satisfactory R^2 value of 0.9658. The response surface plots

showed that the total flavonoid content was maximised with increasing rehydration time. There was no significant effect as temperature was increased. The circular contour plot confirmed a negligible interaction between the variables (Fig. 7.3).

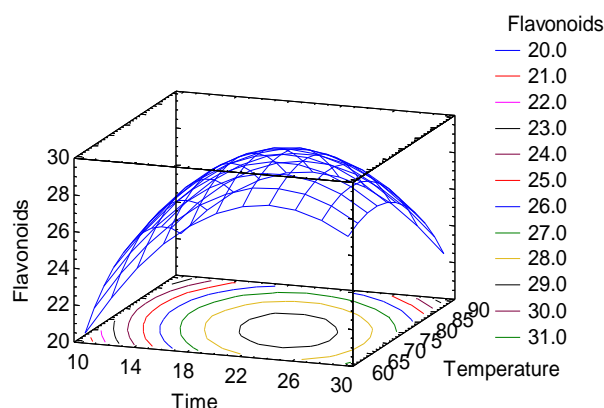


Fig. 7.3 Response surface plot showing effects of rehydration time (min) and temperature (°C) on total flavonoids (mg QE/100 g fw) of *H. elongata*

Flavonoids commonly accumulate in epidermal cells of plant organs, where they are found as glycosides and in non-glycosidic forms (aglycones) (Sakihama *et al.*, 2002). Release of flavonoids and increased chemical extraction of these compounds is inducible by blanching (Olivera *et al.*, 2008). This release of flavonoids coupled with contact and leaching into water may have strongly reduced flavonoid content in samples rehydrated at high temperatures for long durations. The results of the present study are similar to Olivera *et al.* (2008) who found that blanching decreased TFC in brussel sprouts, however these authors found less leaching of flavonoids than the present study (their processing times were significantly shorter). In the present study the flavonoid levels ranged from 13.61 – 6.90 mg QE/100g fw. This represents

up to an 85% loss in flavonoid levels as compared to fresh. This could potentially be due to changes in the cellular structure of seaweed during drying as it becomes quite brittle and upon rehydration phytochemical leaching levels can be quite high.

7.3.5 Effects of process variables on total condensed tannin content

Phlorotannins are a group of phenolic compounds restricted to polymers of phloroglucinol and have been identified from several brown algae. Many studies have shown that phlorotannins are the only phenolic group in brown algae (Jormalainen and Honkanen, 2004 and Koivikko *et al.*, 2007). Tannins can contain some anti-nutritional properties therefore from a food point of view it can be important to avoid excessive consumption of these compounds if at high levels. The model obtained for the total condensed tannin content of rehydrated *H. elongata* was:

$$Z = 2.2226 - 0.527452*X_1 + 0.559293*X_2 + 0.00948114*X_1^2 - 0.00184167*X_1*X_2 - 0.00404377*X_2^2$$

Eq. 7.5

There were significant ($P < 0.05$) influences of X_1 and X_2 on the model. All interaction terms were non-significant ($P > 0.05$). The fit of the model was further confirmed by a satisfactory R^2 value of 0.9528. Tannin levels were as low as 7.48 mg CE/100 g fw, which is almost a 90% reduction from fresh samples. As components of food, tannins reduce the biological value of dietary proteins (Bressani, 1993). Therefore such losses of these antinutritional components can be beneficial. From the response surface plots generated for rehydrated *H. elongata* (Fig. 7.4), it can be

seen that varying time and temperature caused little enhancement or reduction in total condensed tannins as all rehydration time/temperature combinations had a significant effect in the reduction of tannins as compared to fresh seaweed.

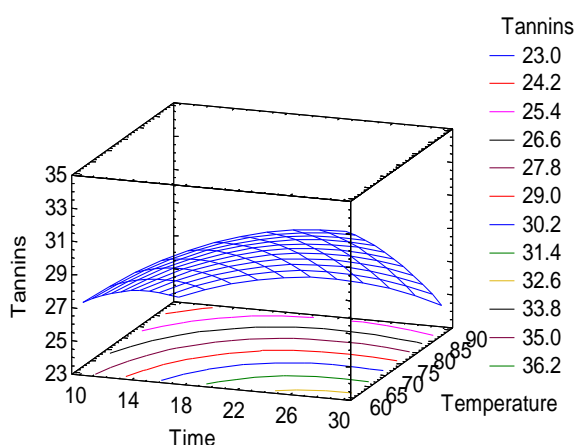


Fig. 7.4 Response surface plot showing effects of rehydration time (min) and temperature (°C) on total condensed tannins (mg CE/100 g fw) of *H. elongata*

7.3.6 Effects of process variables on the texture

Processing time, texture, appearance and flavour are important quality characteristics in the treatment of foods (Xu and Chang, 2008). The texture of dried *H. elongata* is quite tough and rehydration in hot water is often required to make it palatable. Hydrothermal processing times (10 – 30 min) were chosen based on the results of preliminary experiments in which an edible texture was first determined by the tactile method. To overcome the subjectivity of the tactile method, a combination of tactile and instrumental textural methods were used to decide the edible texture of seaweed. From these methods, it was found that a softness of 30 – 32 N/mm was an acceptable edible texture. RSM was used to study texture in order to take into

account the required combination of an edible texture and optimised phytochemical composition.

The model obtained for texture of rehydrated *H. elongata* was:

$$Z = 65.7186 - 1.83977*X_1 - 0.394061*X_2 + 0.0336521*X_1^2 + 0.00439167*X_1*X_2 + 0.00220277*X_2^2 \quad \text{Eq. 7.6}$$

There was a significant ($P < 0.05$) influence of rehydration time, X_1 , and the interaction terms X_1*X_1 (time \times time) in the model (Table 7.3). There was no significant influence of X_2 (temperature) on the model. The fit of the model was confirmed by a satisfactory R^2 value of 0.9174. The response surface plot (Fig. 7.5) showed that the texture softened as the rehydration time increased, but there were no major changes in texture with increasing temperature. This would be due to the fact that as rehydration times are increased, more water is absorbed until a saturation level has been achieved. It may be expected that, as the temperature is increased the rate of water flux would also increase leading to textural degradations. This was not the case in the present study, due to the fact that the seaweed was semi-dried and rehydration times were fast at all water temperatures thus making rehydration times the most important factor for textural degradation.

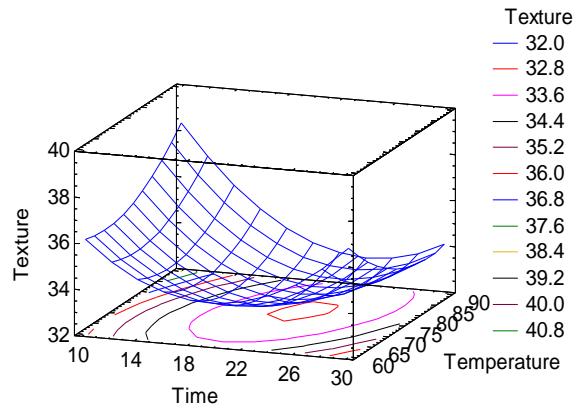


Fig. 7.5 Response surface plot showing effects of rehydration time (min) and temperature (°C) on texture (N/mm) of *H. elongata*

7.3.7 Effects of process variables on the colour

Colour is as an important element in food choice, often reflecting our expectations of flavours (Hutchings, 2003; Lavin and Lawless, 1998 and Leon *et al.*, 1999). Commonly *H. elongata* is dried and during the process, colour darkens from brown to almost black. The seaweed becomes bright green upon rehydration under appropriate conditions, which is an important colour change. The model obtained for colour of hydrothermally processed *H. elongata* was:

$$Z = 15.5294 + 0.101367 \cdot X_1 - 0.293434 \cdot X_2 + 0.00621854 \cdot X_1^2 - 0.00377769 \cdot X_1 \cdot X_2 + 0.00314807 \cdot X_2^2 \quad \text{Eq. 7.7}$$

X_1 and X_2 were significant model terms $P < 0.05$ and there was no significant interaction term in the model (Table 7.3). The R^2 value was 0.9407 which is highly significant. The response surface plot (Fig. 7.6) indicated that the overall colour of seaweed increased (became brighter) as the time and temperature increased; this is

an important colour change as it makes the seaweed more visually attractive. This indicates that both time and temperature of rehydration had a significant effect on the colour change which was expected as the seaweed becomes brighter in colour when exposed to high temperatures and this becomes more pronounced as the time is increased up to a certain time in which the colour change has become completely green.

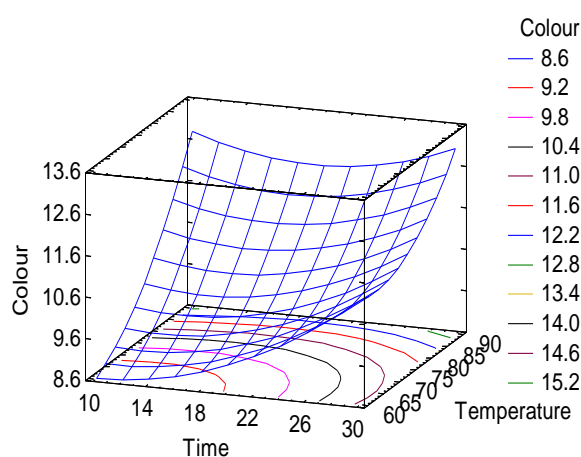


Fig. 7.6 Response surface plot showing effects of rehydration time (min) and temperature (°C) on colour (ΔE) of *H. elongata*

7.3.8 Optimisation

Optimum conditions of rehydration for enhancing each of the bioactive compounds were slightly different. A number of combinations of variables produced maximum total phenolic content, DPPH radical scavenging activity and total flavonoids while still achieving good colour and texture. It was also important to minimise total condensed tannins. As a result, emphasis was placed on optimising the phytochemical constituents (total phenolic content, DPPH activity and total

flavonoids). Optimum rehydration conditions for maximising phytochemical constituents are depicted in Fig. 7.2 below.

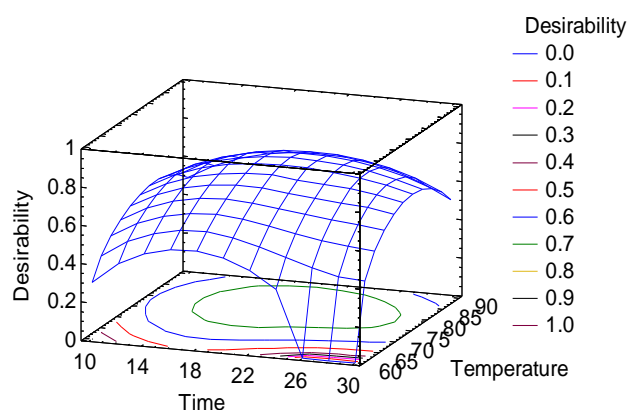


Fig. 7.7 Response surface plot showing optimised effect of rehydration time (min) and temperature (°C) on seaweeds which maximise phytochemical constituents of *H. elongata*

The multi-response analysis of response surface design using the desirability approach was used to optimise rehydration time and temperature. The desirability function is an approach for solving the problem of optimising several responses and is applied when various responses have to be considered at the same time. A desirability function is first constructed for each individual response, and then it is possible to obtain the overall desirability. Multiple response optimisation indicated that phytochemicals in seaweed could be maximised by rehydration for 20.4 minutes at 80.5 °C. This is a promising finding as the time is short and the temperature is lower than boiling (100 °C). The response values predicted under these conditions by the multiple response optimisation were 63.5 mg GAE/100 g fw for TPC, 99.5% for

DPPH radical scavenging activity, 13.2 mg QE/100 g fw for TFC and 12.8 mg CE/100 g fw for TTC. A validation experiment was carried out by rehydrating the seaweed at this optimised time and temperature combination. The phytochemical constituent contents were 61.5 mg GAE/100 g fw for TPC, DPPH radical scavenging activity was 98.9%, TFC was 11.5 mg CE/100 g fw and TTC was 10.2 mg QE/100 g fw. Texture was within the edible range as studied in Chapter 5 at 30.3 N/mm and total colour was 10.5 which indicated a significant and important colour change.

7.4 Conclusion

Response surface methodology using central composite design was demonstrated to be an effective technique for optimising rehydration conditions for the enhancement of phytochemical constituents in semi-dried *H. elongata*. From the response surface plots and statistical analysis, rehydration time was found to have the most significant effect on phytochemical content of the seaweed. This is most likely due to the leaching of phytochemicals which increases with longer rehydration times. Temperature changes also had some significance on the phytochemical levels particularly in the case of TPC as higher temperatures can reduce the bioactivity. The high coefficients of determination of the variables at a 95% confidence level for the six mathematical models developed in this chapter indicated that second order polynomial models could be employed to predict critical phytochemical parameters of *H. elongata* along with texture and colour. Hydrothermal processing is very important in the case of this semi-dried seaweed not only just to rehydrate but to treat the product until it is edible. Therefore it is important that rehydration is optimised so that the duration of treatment is adequate to allow for tenderisation of the seaweed while at the same time minimising phytochemical losses. Such an

optimised product may be applied in the development of new functional foods from *H. elongata* such as incorporation of such seaweeds into existing foods which are commonly consumed.

CHAPTER 8

DEVELOPMENT OF SEAWEED BASED PRODUCTS: INCORPORATION OF SEAWEEDS “*H. ELONGATA*” IN BAKERY PRODUCTS

*Development of new functional foods based on utilisation of Irish edible seaweed *H. elongata**

*The results from this chapter were published as a peer-reviewed article in the
International Food Research Journal*

Summary

Optimisation of seaweed breadsticks was carried out using response surface methodology (RSM). Ten formulations of breadsticks were processed by varying concentrations of seaweed (X_1 = 5 to 15% of overall flour concentration) and white flour (X_2 = 10 to 30% of overall flour concentration) using a central composite design. The remaining flour concentrations were comprised of wholemeal flour. Predicted models were found to be significant ($P < 0.05$) for total phenolic content (TPC), DPPH radical scavenging activity, texture and colour. Predicted values for each of the responses were in good agreement with the experimental values. Seaweed concentration had most significant effect on phytochemical constituents of the breadsticks with TPC and DPPH activity maximised when 17.07% *H. elongata* was incorporated into the flour ($P < 0.05$). An acceptable edible texture and colour of seaweed was also achieved at this concentration. Multiple response optimisation demonstrated that phytochemical content of *H. elongata* breadsticks may be maximised with dried seaweed and white flour concentrations of 17.07 and 21.89%, respectively, in the total flour. Total dietary fibre increased from 4.65 to 7.95% in the optimised sample, representing a 43.65% increase ($P < 0.05$). A group of 20 consumers evaluated the acceptability of the seaweed breadsticks, as compared to the control, in terms of aroma, colour, texture, taste and overall acceptability. It was found that there was no significant difference ($P > 0.05$) between the seaweed breadsticks and the control which shows that such fibre-rich seaweed bakery products are acceptable to consumers and have potential of increasing seaweed consumption among non-seaweed consumers.

8.1 Introduction

Marine food, due to its phenomenal biodiversity is a treasure house of many novel healthy food ingredients and biologically active compounds such as those found in seaweeds. Despite having so many health benefits, marine functional foods have been underexploited for food purposes. Bakery products are widely consumed throughout the world and are the best sources of incorporating marine functional ingredients and reaching the targeted population (Kadam and Prabhasankar, 2010). Currently, the terms ‘functional foods’ and ‘nutraceuticals’ are dominating the food market. Bread is an excellent product in which incorporation of ‘nutraceuticals’ is attempted. One of the latest enrichments has been the addition of omega-3 PUFA to improve essential fatty acid intake. In Europe, consumption of bread enriched with omega-3 PUFA is steadily increasing because Europeans recognise the healthy component of such products. Therefore, the near future for nutrition could potentially include extending the use of breads as vehicles for different micronutrients (Kadam and Prabhasankar, 2010).

Seaweed contains a significant amount of soluble polysaccharides, and has potential function as dietary fibre. The seaweed polysaccharides possess a higher water holding capacity (WHC) than cellulosic fibres. The polysaccharides in seaweeds are sources of hydrocolloids which generally improve the hydration properties and fat holding capacity of products which they are added to, thus reducing fat and water loss during cooking and increasing emulsion stability (Thebaudin *et al.*, 1997; Cofrades *et al.*, 2000; Jiménez-Colmenero *et al.*, 2005). There is an interest in seaweed hydrocolloids for human nutrition as they can act as dietary fibre since their

physiological effects are closely related to their physicochemical properties such as solubility, viscosity, hydration, and ion-exchange capacities in the digestive tract (Lahaye and Kaeffer, 1997). Dietary fibre (DF) is the edible portion of plants (or analogous carbohydrates) which is resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine (Gelroth and Ranhotra, 2001). The term DF comprises polysaccharides, oligosaccharides and associated plant compounds (AACC, 2001).

Brown seaweeds are known to contain more bioactive components than red or green seaweeds (Seafoodplus, 2008). Some of the bioactive compounds identified in brown seaweeds include phylophoeophyllin, phlorotannins, fucoxanthin and various other metabolites (Hosakawa *et al.*, 2006). Such antioxidants from natural sources can be added to products as an ingredient to increase the quality and shelf-life which also considerably enhances the consumer preference (Farak *et al.*, 2003). This along with the benefits of its high fibre shows potential for seaweeds as functional foods; however, in Western countries seaweeds are not widely consumed in diets and are mainly used in alginate production. Therefore any attempt to use seaweeds as a food ingredient should offer better utility of this raw material.

Development of functional foods is currently one of the most intensive areas of food product development worldwide. Product optimisation is an effective strategy to accomplish successful development of the product with respect to a number of attributes. If a food product cannot be re-engineered or modified to fulfil consumer desires and demand for the product, it will not succeed (Robinson, 2000). Therefore response surface methodology (RSM) is often employed for product optimisation

within the food industry. The present study aimed to identify a food-based application for dried edible Irish seaweed in order to encourage consumption amongst non-seaweed eaters. The idea was to scientifically evaluate and improve the quality and nutritional content of a bakery product upon the incorporation of seaweeds. Wheat is the principal cereal used in the preparation of a variety of bakery products, however there is a current trend to move away from white breads towards whole grains such as whole meal flour. Therefore in the present study, the overall flour base mix consisted of varying levels of dried seaweed, white and wholemeal flours.

The main aims of this chapter were as follows:

1. To apply response surface methodology to optimise the dried seaweed and white flour concentrations to develop a new functional bakery product and exemplified in this study as breadsticks.
2. To investigate the effects of seaweed and white flour concentrations on the phytochemical content of breadsticks and to develop a model equation to predict and determine the optimum composition of flour which maximises the photochemical content of seaweed breadsticks.
3. To assess the effect of seaweed and white flour concentrations on the colour and texture of breadsticks.
4. To analyse the effect of seaweed addition on the fibre content of the developed breadsticks.
5. To carry out sensory analysis to determine the acceptability of seaweed breadsticks among consumers.

8.2 Materials and methods

8.2.1 Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in October 2011 and stored at 4 °C until further use.

8.2.2 Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before dehydration.

8.2.3 Dehydration procedure

Dehydration was carried out at 40 °C for 24 hours as described in section 3.12.1. The dried seaweed was then ground into a fine powder using a blender (Rotor).

8.2.4 Experimental design

To investigate the effect of factors (seaweed and white flour concentration) on phytochemical constituents, colour and texture of breadsticks, a central composite design with two factors was utilised. The central composite design was applied using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA, USA). The total number of experiments generated from the software with two factors was 10 ($= 2^k + 2k + 2$), where k is the number of factors. Eight experiments were augmented with two duplicates at the centre points. The level of codes for the independent variables are presented in Table 8.1 below.

Table 8.1 Level of codes for independent variables used in the central composite design

Independent variables	Symbol	-2	-1	0	+1	+2
Seaweed concentration (%)*	X ₁	2.93	5	10	15	17.07
White flour concentration (%)*	X ₂	5.86	10	20	30	34.14

***Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal**

The design matrix and variable combinations of seaweed and white flour concentrations in the experimental runs are shown in Table 8.2. The independent variable concentration applied were seaweed concentration (5 - 15%) and white flour (10 - 30%), with wholemeal flour making up the remaining quantity up to 100%. Therefore as a percentage of the overall recipes of 411g, these values consisted of 1.82 - 10.33 and 3.65 - 20.67% of seaweed and white flour, respectively.

Table 8.2 Design matrix and variable combinations in experimental runs

Experiment	Seaweed concentration (%)*	White flour concentration (%)*
1	15.00	10.00
2	10.00	20.00
3	5.00	30.00
4	10.00	20.00
5	17.07	20.00
6	10.00	5.86
7	5.00	10.00
8	2.93	20.00
9	10.00	34.14
10	15.00	30.00

***Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal**

Experimental data from the central composite design was analysed and fitted to a polynomial regression model below:

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_{11} \chi_1^2 + \beta_{22} \chi_2^2 + \beta_{12} \chi_1 \chi_2 \quad \textbf{Eq. 8.1}$$

Where; Y is response calculated by the model: β_0 is a constant and β_i , β_{ii} and β_{ij} are linear, squared and interaction coefficients, respectively.

A multi-response analysis of the response surface design was performed using the desirability approach to optimise seaweed and white flour concentrations. The methodology is based on first constructing a desirability function for each individual response, and then the overall desirability is obtained.

8.2.5 Seaweed breadstick preparation

Seaweed and flour blends were prepared by the replacement method according to the RSM experiment. The percentages of seaweed and white flour from the RSM (Table 8.2) are based on percentages of overall flour in the mix (flour consisted of 60.79% of the mix), with wholemeal flour comprising the remaining component of the mix. The concentrations of ingredients for each of the experiments can be seen in Table 8.3. Firstly, the yeast was dissolved in the water and added to the dry ingredients (except seaweed). The ingredients were mixed at slow speed for 2 min, then at medium speed for 4 min (Hobard A120 mixer, Hobard MFG Co. Ltd, London, UK). Seaweed was then added and mixed again for a further 2 min. The dough was placed on trays and left to develop for 45 min then moulded into breadstick shapes by hand and proofed in a proofer (Sveba Dahlen, Sveba Dahlen, Fristan, Sweden) at 33 °C, 78% RH for 40 min. The breadsticks were then baked in an oven (Sveba Dahlen, DC 44, Sveba Dahlen, Fristan, Sweden) at 210 °C for 20 min with 10 seconds of steam at the beginning.

Table 8.3 Experimental design for seaweed breadsticks

Experiment	Seaweed (%)	White flour (%)	Wholemeal flour (%)	Salt (%)	Butter (%)	Yeast (%)	Water (%)
1	9.12	6.08	45.59	1.21	1.21	2.13	34.65
2	6.08	12.16	42.55	1.21	1.21	2.13	34.65
3	3.04	18.24	39.51	1.21	1.21	2.13	34.65
4	6.08	12.16	42.55	1.21	1.21	2.13	34.65
5	10.33	12.16	38.30	1.21	1.21	2.13	34.65
6	6.08	3.65	51.06	1.21	1.21	2.13	34.65
7	3.04	6.08	51.67	1.21	1.21	2.13	34.65
8	1.82	12.16	46.81	1.21	1.21	2.13	34.65
9	6.08	20.67	34.04	1.21	1.21	2.13	34.65
10	9.12	18.24	33.43	1.21	1.21	2.13	34.65

8.2.6 Extraction of phytochemicals

Seaweed and breadstick samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted as described in section 3.2.

8.2.7 Total phenolic content

The total phenolic content of seaweed samples was measured using the Folin-Ciocalteu method (Taga *et al.*, 1984) as described in section 3.4. The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram dry basis (db) (mg GAE/100 g db).

8.2.8 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as described in section 3.7. Results were expressed as % reduction in DPPH radical scavenging activity.

8.2.9 Texture evaluation

Shear tests were performed on breadsticks as described in section 3.9.

8.2.10 Colour measurement

Colour analysis was carried out using a colourimeter (CIE Lab ColourQuest XE, Hunter Associates, Reston, VA, USA) as described in section 3.10.

8.2.11 Total Dietary Fibre

Total dietary fibre was calculated as described in section 3.11.

8.2.12 Sensory characteristics

The consumer acceptance test was conducted in a standardised sensory test room (ISO 8589, 2007). Untrained panelists ($n = 20$) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (20 g) were served on white paper plates with random three-digit numbers and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall

acceptability of the product (maximum of 5), where 5 was “like extremely” and 1 was “dislike extremely”. The overall quality (maximum of 25) was computed by combining scores of all five attributes.

8.2.13 Statistical analysis

All experiments were carried out in triplicate and replicated at least twice. Data from the central composite design were subjected to a second-order multiple regression analysis using least-squares regression to obtain the parameter estimated for the mathematical model. The regression analysis and analysis of variance (ANOVA) were performed with the STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). Differences were considered statistically significant when $P < 0.05$.

8.3 Results and Discussion

8.3.1 Statistical analysis of results obtained by experimental design

The effect of a range of drying temperatures on the drying kinetics and phytochemical constituents of *H. elongata* was investigated and results showed that drying was optimised at 40 °C and therefore these drying conditions were applied in the current study (Gupta *et al.*, 2011). The rationale behind adding seaweed to breadsticks was based on the fact that bakery products are widely consumed; therefore addition of *H. elongata* would widen the consumer base and would further improve the nutraceutical properties of this product. Breadsticks were investigated as a means of seaweed incorporation as the texture of both breadsticks and the dried seaweed in the current study are generally tough thus masking the chewiness of the

dried seaweed. Dried seaweed is also convenient and cost effective as volumes are reduced thus lowering transport costs and therefore considered a viable ingredient to add value to existing products.

Preliminary experiments were carried out in order to determine the maximum levels of seaweed which could be added to the breadsticks with respect to texture and flavour. Higher seaweed concentrations ($\geq 20\%$) led to unacceptable end products as the baked product was quite tough and difficult to chew. Once the maximum level of seaweed was established at 15%, RSM was applied as it is a useful tool for optimising functional ingredients within products. It has been used in various food and drink product development processes (Deshpande *et al.*, 2008; Wadikar *et al.*, 2008; Villegas *et al.*, 2010). In this study, ten experiments were performed to determine the optimum concentrations of seaweed and flour blends required to maximise the phytochemical level in breadsticks. The effects of independent variables (seaweed and white flour concentrations) for each of the response variables (TPC, DPPH, texture and colour) are presented in Table 8.4.

Table 8.4 Two-way ANOVA for the independent variables on the response of total phenolic content, DPPH, texture and colour of seaweed breadsticks

Source	Total phenolic content		DPPH		Texture		Colour	
	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -value
X ₁	762.40	0.0000	66.82	0.0012	2020.32	0.0000	1.50	0.2874
X ₂	0.11	0.7548	0.12	0.7464	2.17	0.2145	0.22	0.0345
X ₁ *X ₁	3.13	0.1515	4.65	0.0973	74.44	0.0010	9.93	0.0345
X ₁ *X ₂	0.09	0.7760	0.06	0.8192	0.00	0.9829	2.06	0.2242
X ₂ *X ₂	0.03	0.8812	2.11	0.2203	0.23	0.6579	3.40	0.1390
<i>R</i>² values: 0.9948 (total phenolic content), 0.9973 (DPPH), 0.9981 (texture) and 0.7780 (colour).								

The models for each of the responses were analysed separately before overall optimum seaweed and flour concentrations for the breadstick recipe were determined. Predicted and experimental values for each of the responses are presented in Table 8.5 and were in good agreement with the experimental values. Response surface plots were generated to illustrate the effects of blanching time and temperature on each of the responses (Fig. 8.1 – 8.4).

Table 8.5 Predicted (Pred.) and experimental (Exp.) values of total phenolic content, DPPH, texture and colour of seaweed breadsticks

Experiment No.	Total phenolic content (mg GAE/100g db)		DPPH (%)		Texture (N/mm)		Colour (ΔE)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	118.02	122.02	60.25	60.50	99.36	99.99	11.85	11.75
2	78.99	77.33	52.18	57.08	81.51	81.82	19.17	18.39
3	38.99	40.30	40.44	40.76	70.90	71.14	5.47	8.50
4	75.66	77.33	61.98	57.08	82.12	81.82	17.6	18.39
5	145.88	142.84	65.24	64.46	108.84	108.66	12.79	12.07
6	80.16	75.99	51.36	51.62	83.82	82.86	14.35	14.36
7	34.55	38.01	41.21	40.76	71.05	72.04	12.34	13.29
8	28.11	25.84	35.11	35.32	69.85	69.16	10.96	8.76
9	78.54	77.40	53.69	52.86	81.47	81.56	16.02	13.08
10	119.88	121.74	61.22	62.24	99.17	99.05	12.75	14.73

Values are presented as mean (n = 6).

8.3.2 Effects of variables (seaweed and white flour concentrations) on total phenolic content of the breadsticks

Experimental results for total phenolic content (TPC) were fitted to a full quadratic second order polynomial equation and the model obtained for TPC of the breadsticks was:

$$Z = 3.77979 + 5.72532 * X_1 + 0.305353 * X_2 + 0.140273 * X_1^2 - 0.0129 * X_1 * X_2 - 0.00315601 * X_2^2 \quad \text{Eq. 8.2}$$

(See Table 8.1 for definitions of X_1 and X_2). In order to determine the significance of the model, ANOVA was carried out on the data. The F -value for seaweed concentration (X_1) was high (762.40) indicating that this factor was highly significant (Table 8.4). All other interaction factors and white flour concentration (X_2) had low F -values which suggest that TPC had mainly resulted from the addition of seaweed. The model explained 99.48% (R^2 of 0.9948) of the variation in TPC which is quite significant. This indicates that only 0.52% of the variation in TPC was due to factors not included in the model.

The P -values were used to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the P -value, the larger the significance of the corresponding coefficient is. P -values indicated that, among the test variables and their interactions, X_1 (seaweed concentration) was highly significant ($P < 0.05$) but all other factors; X_2 (white flour concentration), $X_1 * X_1$ (seaweed concentration \times seaweed concentration), $X_1 * X_2$ (seaweed

concentration \times white flour concentration) and X_2^2 (white flour concentration \times white flour concentration) were insignificant model terms with P -values > 0.05 .

The polynomial response models were expressed as three-dimensional (3D) surface plots to better visualise the relationship between the seaweed and white flour concentrations as independent variables and phytochemical properties as response variables. The fitted response surface plots were generated using Statgraphics software to illustrate the interaction of parameters required for optimum responses. The response plot (Fig. 8.1) showed that TPC increased sharply with increasing seaweed concentration ($P < 0.05$), while TPC remained unchanged with increasing white flour concentration as observed in Table 8.4.

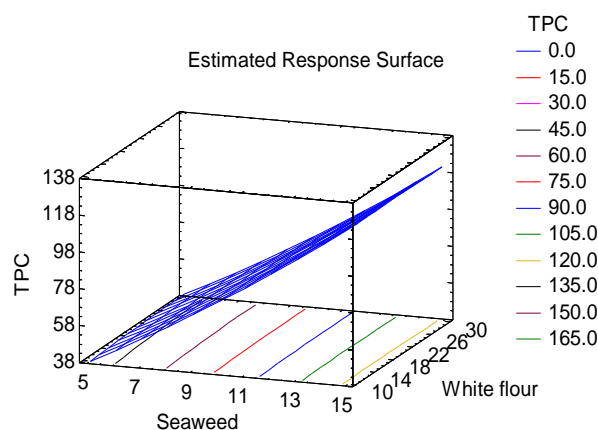


Fig. 8.1 Response surface plots showing effects of seaweed and white flour concentrations (%) on the total phenolic content (GAE/100 g db) of seaweed breadsticks

The addition of seaweed to the breadsticks significantly increased the TPC ($P < 0.05$). An 81.03% increase was seen when the overall flour concentration was substituted with 17.07% seaweed. These results are higher than those reported for other cereal based food products which were incorporated with seaweed. Prabhasankar *et al.* (2009) studied the influence of adding brown seaweed, *Sargassum marginatum*, to pasta. The TPC in cooked pasta increased from 9 to 13 mg GAE/100 g with 5% addition of the brown seaweed. Although the previous study showed that phenolics leached into cooking water, these results are still significantly lower than those of the present study. Comparing with the same seaweed concentration, the results of 5% incorporation of seaweed in breadsticks increased the TPC from 27.67 to 38.99 mg GAE/100 g db which is also higher than that of Prabhasankar *et al.* (2009).

The breadsticks containing maximum *H. elongata* concentration (17.07%) showed an increase in the TPC from 27.67 to 145.88 mg GAE/100 g db which is an increase of 81.03% as compared to the control. Prabhasankar *et al.* (2009) also reported that an addition of 30% *Undaria pinnatifida* seaweed increased the TPC of pasta from 9 – 27 mg GAE/100 g. Again, this is considerably less than obtained in the present study. TPC of bread samples with different percentages of ginger powder were studied by Balestra *et al.* (2011). TPC levels increased from 14.30 to 48.50 GAE/100 g db with 6% addition of ginger powder. This clearly shows that the seaweed breadsticks had higher levels of total phenols compared to that of other nutraceutical cereal based products such as bread and pasta.

8.3.3 Effects of variables (seaweed and white flour concentrations) on DPPH radical scavenging activity of the breadsticks

The mathematical model obtained for the DPPH radical scavenging activity of the breadsticks was:

$$Z = 13.2787 + 4.76275 * X_1 + 0.92469 * X_2 - 0.1438 * X_1^2 + 0.0087 * X_1 * X_2 - 0.0242 * X_2^2$$

Eq. 8.3

There was a significant ($P < 0.05$) influence of the linear factor of X_1 (seaweed concentration) on the model. The linear factor of X_2 (white flour concentration) and all quadratic factors and interactions $X_1 * X_1$ (seaweed concentration \times seaweed concentration), $X_1 * X_2$ (seaweed concentration \times white flour concentration) and $X_2 * X_2$ (white flour concentration \times white flour concentration) were insignificant model terms with P -values > 0.05 in terms of DPPH radical scavenging activity. This showed that seaweed concentration had the greatest impact on the DPPH radical scavenging activity of the breadsticks which was expected as seaweed exhibit high levels of DPPH radical scavenging activity. The fit of the model was further confirmed by a high coefficient of determination, 0.9973 meaning that 99.73% of the variation in DPPH activity was explained by the model. The response surface plots generated showed that DPPH radical scavenging activity increased with increasing seaweed concentration while the activity remained more or less constant with respect to the effect of white flour concentration (Fig. 8.2). The lack of significance of the white flour concentration on the DPPH activity of the breadsticks is further confirmed by the circular shape of the contour plots which indicates that the interactions are negligible.

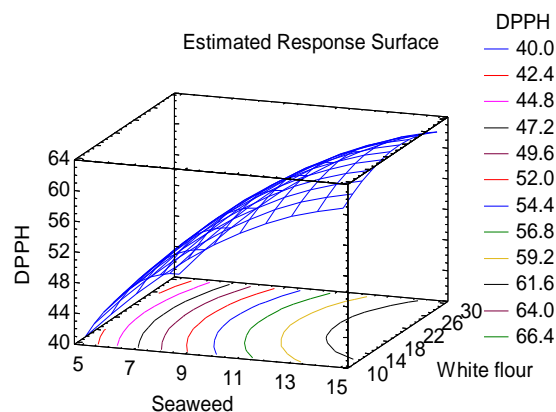


Fig. 8.2 Response surface plots showing effects of seaweed and white flour concentrations (%) on DPPH radical scavenging activity (%) of seaweed breadsticks

The DPPH radical scavenging activity of the control breadsticks (containing no seaweed) was 34.81%. Replacement of flour with 17.07% seaweed increased the DPPH activity to 65.24%, representing a significant increase of 46.64% in DPPH activity ($P < 0.05$). Any level of seaweed above 5% significantly increased the DPPH activity of the seaweed breadsticks ($P < 0.05$). Balestra *et al.* (2011) also found a significant increase in DPPH activity with the addition of 6% ginger powder to breads (86.75% increase). In seaweed incorporated pasta, it was found that addition of 30% brown seaweed increased the DPPH activity from 6.83 to 9.79% (Prabhasankar *et al.*, 2009) which is significantly lower than the activity in the present study. In chapter 5 it is reported that dehydration can lead to slight decreases in DPPH activity but thermal processing such as boiling, applied after drying can lead to significant increases in the activity. It is possible that the temperature upon

baking of the breadsticks could also have increased the DPPH radical scavenging activity of extracts from the final product. This indicates that addition of *H. elongata* seaweed to breadsticks would provide a good source of antioxidants.

8.3.4 Effects of variables (seaweed and white flour concentrations) on the texture of the breadsticks

For a novel food product, it is necessary to study the impact of added ingredients on food quality attributes. Hardness or firmness is an important factor in the quality of breadsticks. The texture of dried *H. elongata* is quite hard and processing is often required to make it more palatable. Common food processing methods such as boiling can lead to loss of phytochemicals (Cox *et al.*, 2011). To overcome the issues with the noticeable hardness of dried *H. elongata*, the dried seaweed was ground into a powder. The seaweed powder was then incorporated into breadsticks. The model obtained for texture of the breadsticks was:

$$Z = 69.7308 - 0.0399788 * X_1 - 0.122297 * X_2 + 0.141849 * X_1^2 - 0.0002 * X_1 * X_2 + 0.0019626 * X_2^2 \quad \text{Eq. 8.4}$$

There was a significant ($P < 0.05$) influence of seaweed concentration, X_1 , and the quadratic terms $X_1 * X_1$ (seaweed concentration \times seaweed concentration) on the model (Table 8.3). However, there was no significant influence of white flour concentration (X_2) or the quadratic term $X_2 * X_2$ (seaweed concentration \times seaweed concentration) or interaction term $X_1 * X_2$ (seaweed concentration \times white flour concentration) on the model. The fit of the model was confirmed by a satisfactory R^2 value of 0.9981 which is very high. The response surface plot (Fig. 8.3) showed that

the texture became harder with increasing seaweed concentration, but there were no major changes in hardness with increasing white flour concentration which was expected.

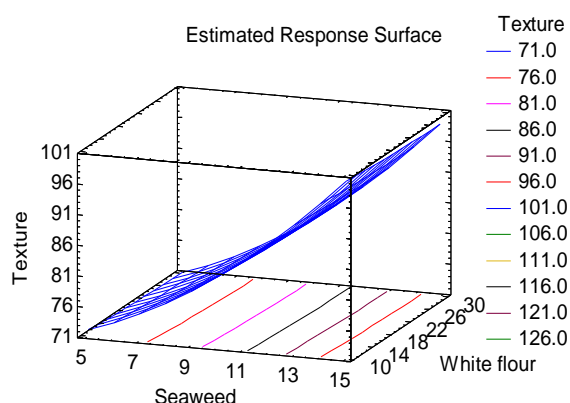


Fig. 8.3 Response surface plot showing effects of seaweed and white flour concentrations (%) on the texture (N/mm) of seaweed breadsticks

The hardness of the control breadsticks was calculated as 74.38 N/mm using an Instron texture analyser, and fortification of flour with seaweed at all levels (2.93 to 17.07%) significantly increased the hardness of the breadsticks ($P < 0.05$). Hardness was maximised in the present study, when flour was replaced with 17.07% seaweed (108.84 N/mm). Prabhasankar *et al.* (2008 and 2010) also found that adding seaweed to pasta (1 – 5%) increased the firmness of the product. Chang and Wu (2008) added 4 – 8% green seaweed to noodles and also found that there was an increase in the hardness with increasing seaweed concentration. However, in order to determine if such an increase was acceptable to the consumer, the texture of the breadsticks was taken into account in the sensory analysis study (section 8.3.8).

8.3.5 Effects of variables (seaweed and white flour concentrations) on the colour of the breadsticks

Commonly *H. elongata* is dried and during the dehydration process, colour darkens from brown to almost black (Cox *et al.*, 2012). Colour is an important characteristic for baked products because together with texture and aroma, it contributes to consumer preference. It is dependant on physicochemical characteristic of the dough (water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking (temperature, relative humidity, modes of heat transfer) (Esteller and Lannes, 2008). The consumer understanding of the expected colour of baked goods is well known and this characteristic colour would be expected with new baked products. The model obtained for colour change of breadsticks with added seaweeds was:

$$Z = -0.562436 + 2.64694 * X_1 + 0.499152 * X_2 - 0.159474 * X_1^2 + 0.03885 * X_1 * X_2 - 0.0233189 * X_2^2 \quad \text{Eq. 8.5}$$

Colour analysis of the breadsticks indicated that the linear factor of seaweed concentration (X_1) had an insignificant effect on the colour of the breadsticks ($P > 0.05$) however the quadratic factors of seaweed concentration ($X_1 * X_1$) were significant ($P < 0.05$). X_2 (white flour concentration) also had a significant ($P < 0.05$) effect on the colour of the breadsticks. There was no significant interaction of the quadratic term $X_2 * X_2$ (white flour concentration \times white flour concentration) or interaction term $X_1 * X_2$ (seaweed concentration \times white flour concentration) on the model ($P > 0.05$) and the R^2 value obtained was 0.7780. This indicated that both seaweed and white flour concentrations had some influence on the colour of the

breadsticks. This was further confirmed by the response surface plot (Fig. 8.4) as it had a spherical response surface which indicated that colour change increased with increasing seaweed concentration but then gradually decreased, while white flour concentration also affected colour change as it increased slightly with increasing flour concentration but then also decreased slightly.

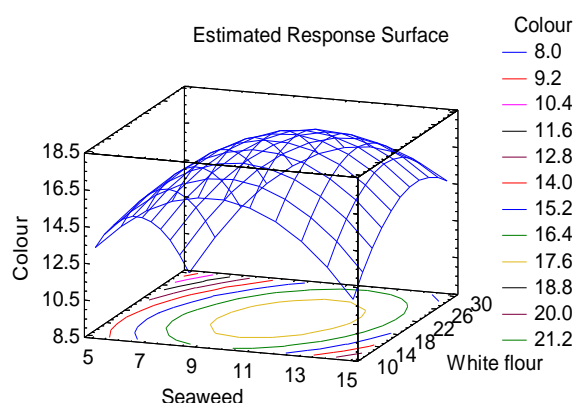


Fig. 8.4 Response surface plots showing effects of seaweed and white flour concentrations (%) on the colour (ΔE) of seaweed breadsticks

The colour change of all samples was significantly different ($P < 0.05$) indicating that the different flour blends with varying concentrations of seaweed, white and wholemeal flour had a significant effect on the colour of the breadsticks. This was expected as the colour of the seaweed is quite dark so varying the seaweed concentrations in the flour from 2.93 to 17.07% would obviously cause a difference in overall colour of the baked breadsticks. In order to determine if the colour of the seaweed breadsticks were acceptable to consumers as compared to the control, colour was taken into account in the sensory evaluation as discussed in section 8.3.8.

8.3.6 Optimisation

Optimum conditions of seaweed and flour concentrations in breadsticks were determined to obtain maximum phytochemicals and enhance dietary fibre as the rational was to develop a functional food product. As the texture (hardness) and colour of the breadsticks were acceptable throughout the ten experiments, they were not included as factors in the optimisation. These factors (texture and colour) were sensorially evaluated by a panel of consumers to determine acceptability. The second order polynomial models obtained in this study for TPC and DPPH responses were utilised in order to determine the specified optimum conditions. Optimum seaweed and white flour concentrations for maximising phytochemical constituents are depicted in Fig. 8.5.

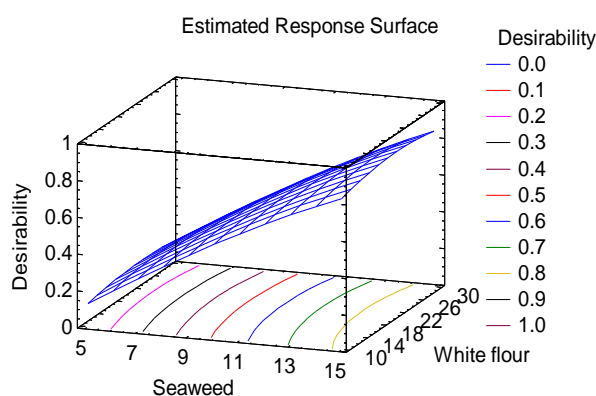


Fig. 8.5 Response surface plot showing optimised effect of seaweed and white flour concentrations (%) to maximise phytochemical constituents of breadsticks By applying the desirability function method (an approach for solving the problem of optimising several responses which have to be considered at the same time) the concentrations were obtained for the breadsticks with optimum phytochemical level. Multiple response optimisation indicated that phytochemicals in breadsticks could be

maximised with 17.07% seaweed and 21.89% white flour concentrations in the overall flour. The response values predicted under these conditions by the multiple response optimisation were 142.75 mg GAE/100 g db for TPC and 64.58% for DPPH radical scavenging activity. A validation experiment was carried out by preparing breadsticks with the optimised dried seaweed and white flour concentrations. The phytochemical constituent contents were 138.25 mg GAE/100 g db for TPC and 65.01% for DPPH radical scavenging activity.

8.3.7 Total dietary fibre

In view of the therapeutic potential of dietary fibre, more fibre incorporated food products are being developed. Fig. 8.6 shows the total dietary fibre (TDF) content of the breadsticks. Dried seaweed contained 39.56% TDF, control breadsticks had 4.65% TDF and the seaweed breadsticks as optimised using RSM (17.07% seaweed added) contained 7.95% TDF which represents a 43.65% increase in the total dietary fibre when compared to breadsticks with no added seaweeds. Addition of seaweed significantly increased the TDF of the breadsticks as compared to the control ($P > 0.05$). These results are higher than those reported in the literature for final products containing seaweed. Prabhasankar *et al.* (2008) developed a seaweed pasta which had 4% fibre, but the amount of seaweed added was considerably less (2.5%). Cofrades *et al.* (2008) found that the addition of 5% *H. elongata* to meat systems only contributed 2.52% TDF to the final product. The same authors also found that the incorporation of *Porphyra umbilicalis* seaweeds at 5%, only fortified meat products with 1.77% fibre. The effect of enrichment of bread with rice bran fibre was studied by Hu *et al.* (2009) and addition of up to 6% rice bran fibre resulted in 4.98% TDF in the final product. Therefore, in the current study, the optimised breadsticks

had a higher TDF in the final product (7.95%), this higher level would also be due to the fact that more seaweed could be added to the breadsticks then to the products in the other studies outlined in literature.

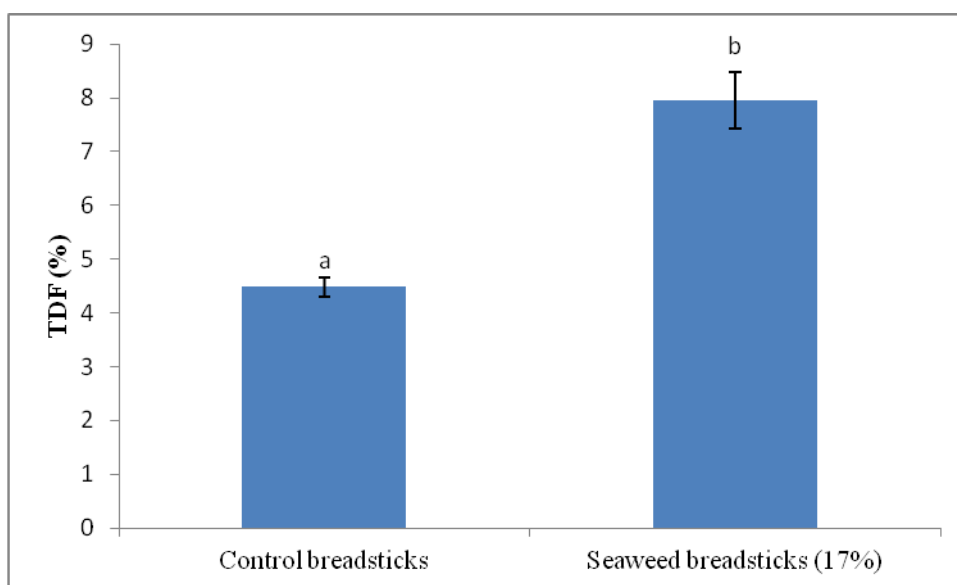


Fig. 8.6 Total dietary fibre content of control and seaweed breadsticks

Each value is presented as mean \pm SD (n = 3).

Means above each bar with different letters (a-b) differ significantly ($P < 0.05$).

8.3.8 Sensory analysis

Table 8.6 summarises the sensory scores for aroma, appearance, texture, taste and overall acceptability of control and seaweed breadsticks. When developing functional bakery products, it is important to design a product with physiological effectiveness that will be accepted by consumers in terms of appearance, taste and texture (Siró *et al.*, 2008). The samples tested by the sensory panel in this study were the control (with no added seaweed), breadsticks with 10% of the flour replaced with seaweed (6.08% concentration of seaweed overall) and the optimised sample from the RSM study which would have the maximum level of antioxidants (17.07%

seaweed in overall flour blend or 10.33% seaweed in the final product). Each of the breadsticks can be seen in Fig. 8.7.



Fig. 8.7 Image of control (a) 10% (b) and (c) 17.07% seaweed breadsticks

Aroma, appearance, texture and taste were found to be significantly different to the control breadsticks ($P > 0.05$). Although there was a significant difference, the scores for each of the seaweed breadsticks were only slightly lower than that of the control, and all three breadsticks were at acceptable values suggesting potential incorporation of seaweeds in bakery products.

Table 8.6 Mean scores for aroma, appearance, texture and taste of the control and seaweed breadsticks

Breadsticks	Sensory attributes				
	Aroma	Appearance	Texture	Taste	Overall acceptability
Control	4.35±0.81a	4.40±0.50a	3.95±0.75a	3.8±0.61a	3.75±0.71a
10% seaweed	3.80±0.61b	3.30±0.92b	3.40±0.94b	3.50±0.68b	3.55±0.68b
17.7% seaweed	3.25±1.06c	3.30±0.92c	3.55±0.94c	2.75±0.85c	2.80±0.76c

Each value is presented as mean ± SD (n = 20).

Means within each column with different letters differ significantly ($P < 0.05$).

The results of the present study are promising as some food products with added fibre are often rated as unacceptable by sensory panels once they exceed a certain concentration. For example, Hu *et al.* (2008) found that the addition of rice bran fibre above 4% was unacceptable by consumers. Also, Prabhasankar *et al.* (2009) found that there was a significant difference in pasta with 10% replacement of semolina with seaweed as compared to the control ($P > 0.05$). This indicates that breadsticks are a good product for seaweed incorporation at high levels without affecting the overall quality of the product.

8.4 Conclusion

Response surface methodology using central composite design was demonstrated to be an effective technique for optimising *H. elongata* and white flour concentrations for enhancement of phytochemical constituents in seaweed breadsticks. From the response surface plots, seaweed concentration was found to have the most significant effect on phytochemical content of the breadsticks. The high coefficients of determination of the variables at a 95% confidence level indicated that second order polynomial models could be employed to predict critical phytochemical parameters of breadsticks containing *H. elongata* along with texture and colour. These breadsticks would provide the consumer with higher levels of dietary fibre (7.95%) and phytochemicals (TPC: 138.25 mg GAE/100 g db; DPPH: 65.01%) and have an appealing colour and texture. There was a significant difference found in the sensory scores for seaweed breadsticks as compared to the control ($P > 0.05$), however all scores were at acceptable levels which is promising.

CHAPTER 9

DEVELOPMENT OF SEAWEED BASED PRODUCTS: INCORPORATION OF SEAWEEDS “*H. ELONGATA*” IN MEAT PRODUCTS

*Development of new functional foods based on utilisation of Irish edible seaweed *H. elongata**

*The results from this chapter were published as a peer-reviewed article in the
International Journal of Food Science and Technology*

Summary

The effect of adding *H. elongata* seaweed (10 – 40% w/w) as a source of antioxidants and dietary fibre on physical, chemical, microbial and sensory traits of cooked beef patties was studied throughout chilled storage (30 days at 4°C). Patties with seaweed showed reduced cooking losses and had a more nearly 50% more tender in texture as compared to patties without seaweed ($P < 0.05$). Microbiological counts and lipid oxidation analysis were significantly lower in patties containing seaweed ($P < 0.05$) and by day 30 of storage there was no bacterial growth recorded on the samples with $\geq 20\%$ seaweed w/w and lipid oxidation levels were low (0.61 mg malondialdehyde/kg of sample). The incorporation of seaweed significantly increased the total dietary fibre (1.64 g per 100 g fw in 40% seaweed-patties), total phenolic content (up to 28.11 mg GAE/100 g fw) and DPPH radical scavenging activity (up to 52.32%) of the patties as compared to the control ($P < 0.05$). Results of sensory analysis indicated that the seaweed-patties were accepted by consumers in terms of aroma, appearance, texture and taste. Patties containing 40% seaweed were rated highest in terms of overall acceptability, most likely due to the improvement in texture and mouthfeel. These results showed that addition of up to 40% seaweed in the formulation of beef patties leads to the enhancement of the nutritional (higher fibre and phytochemical contents) and technological quality (higher cooking yields, more tender texture, enhanced storage life) together with an acceptable sensory quality.

9.1 Introduction

The realisation of the relationship between diet and health is leading to new insights into the effect of food ingredients on physiological functions and health, inducing consumer demand for healthy, nutritious foods with additional health promoting functions (Jiménez-Colmenero *et al.*, 2010). Many new products have been developed and marketed, offering increased health benefits and the potential to reduce the risk of diseases. Sales of such “functional foods” in Europe have increased significantly (Annunziata and Vecchio, 2011). Many components may be added to meat, dairy, fish or vegetable-based products to make them “functional”, such as ω -3 fatty acids, prebiotics, probiotics, fibre or phytochemicals (Jiménez-Colmenero, 2007).

Over the past few decades, meat products have come under increasing scrutiny by medical, nutritional and consumer groups because of the associations established between their consumption (or that of a number of their constituents, such as fat and cholesterol) and the risk of some of the major degenerative and chronic diseases (heart disease, cancer, hypertension and obesity). Therefore meat-based functional foods are being seen as an opportunity to improve the “image” of meat and address consumer nutritional and dietary needs (Jiménez-Colmenero, 2007). As meat is one of the most commonly-consumed foods, it offers an excellent way of promoting intake of functional ingredients without any radical changes in eating habits (Cofrades *et al.*, 2008). This situation is prompting the emergence of new “healthier” meat products. Most physiologically active substances come from plants, and when combined with other foods such as meat, they can help provide a food with

functional effects. Various types of plant ingredients have been used for their technological, sensory, economic and nutritional effects in the meat industry (Jiménez-Colmenero, 2010).

Meat is low in dietary fibre, therefore addition of ingredients containing fibre to common meat products such as patties commonly known as burgers would be beneficial. Dietary fibre intake provides many health benefits such as reducing the risk of developing diseases including coronary heart disease, stroke, hypertension, diabetes, obesity and certain gastrointestinal disorders. Furthermore, increased consumption of dietary fibre improves serum lipid concentrations, lowers blood pressure, improves blood glucose control in diabetes, promotes regularity, aids in weight loss and appears to improve the immune function (Anderson *et al.*, 2009).

Seaweeds are known to be a good source of dietary fibre (Cofrades *et al.*, 2008). Hydrocolloids in seaweeds act as dietary fibre and also improve the hydration properties of foods in which they are added to, therefore leading to reductions in fat and water losses during cooking as emulsion stability is increased (Thebaudin *et al.*, 1997; Cofrades *et al.*, 2000; Jiménez-Colmenero *et al.*, 2005). Plant biomass or its derived bioactive compounds have been considered as possible functional components in processed meat products for alleviation of the colorectal cancer risk associated with the consumption of processed meats (Demeyer *et al.*, 2008). The introduction of functional ingredients such as botanicals, plant extracts and seaweeds with probable biological activity into processed meat products is receiving abundant attention (Calvo *et al.*, 2008; Cofrades *et al.*, 2008; Hayes *et al.*, 2005; Hernández-Hernández *et al.*, 2009; Valencia *et al.*, 2008). Seaweeds are also high in

phytochemicals such as phenolic compounds (Cox *et al.*, 2011). It has been reported that 34% of men and 21.9% of women consume burgers in Ireland (Duffy *et al.*, 2005), therefore incorporation of seaweed into such beef patties would have potential as a means of developing a healthier meat product while exploiting the technological benefits of the hydrocolloids such as increase in dietary fibre and reductions in processing losses.

The main aims of this chapter were as follows:

1. To investigate the effects of adding seaweed at varying concentrations on the phytochemical content of beef patties in order to develop new meat functional foods.
2. To analyse the effect of seaweed incorporation on the physical (cooking yield, fibre, texture and colour) and quality characteristics (bacterial enumeration, pH and lipid oxidation) over the shelf life of beef patties.
3. To carry out sensory analysis to determine the acceptability of seaweed-patties within a panel of meat consumers.

9.2 Materials and methods

9.2.1 Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in October 2011 and stored at 4 °C until further use.

9.2.2 Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before dehydration.

9.2.3 Dehydration and hydrothermal processing procedure

Dehydration was carried out at 40 °C for 2 hours as described in section 3.12.1. These conditions were chosen on the basis of optimisation studies in chapter 6. The dried seaweed was then rehydrated as described in section 3.12.2 with some modifications. The rehydration or blanching conditions were carried out at 80.5 ± 0.5 °C for 20 ± 0.05 min as optimised in chapter 7. The rehydrated seaweed were then ground using a blender (Rotor, Germany) and stored at 4 °C until further usage.

9.2.4 Seaweed-patty preparation

Five different patty formulations were prepared containing 0, 10, 20, 30 and 40% blanched seaweed. Lean beef ($\leq 5\%$ fat) was purchased from a local supermarket and stored immediately in a refrigerator at 4 °C. Meat was cut into smaller pieces using a sterile knife and ground in a meat grinder (Meteor MATR, Ireland) which had been previously sterilised and chilled (4 °C). The seaweed was added to each of the mixtures in sterile bowls (Fig. 9.1) and mixed by hand with sterile utensils until the seaweed was homogenous throughout the meat. The final temperature of the meat was < 12 °C in all cases and was formed with a manual circular shaped mould. The patties were 1 cm thick and weighed 50 ± 0.05 g. Samples were cooked in an oven (Rational Combi, Dämpfer, United Kingdom) at 200 °C for 15 mins. It was ensured that the centre of the patties were ≥ 70 °C for over 2 minutes. The patties were then immediately cooled to 4 °C and placed in polyethylene bags (PA/PE, Brodericks

Brothers Limited, Ireland) and vacuum packed (La Minerva, Italy). The samples were stored at 4 °C throughout the storage period for 30 days which is typical for a cooked beef product.



Fig. 9.1 Image of minced beef and seaweed in sterile bowl before mixing

9.2.5 Cooking properties measurement

Patties were weighed before cooking and after chilling at 4 °C. To estimate the cooking yield, the patty weights were expressed as a percentage of the initial weight using the following calculation:

$$\text{Cooking yield (\%)} = 100 \times \frac{\text{cooked weight (g)}}{\text{raw weight (g)}} \quad \text{Eq. 9.1}$$

9.2.6 Total Dietary Fibre

Total dietary fibre was calculated as described in section 3.11.

9.2.7 Bacterial enumeration

Samples were prepared in a vertical laminar-flow cabinet (Model) for the purposes of microbial analysis. For each patty sample, 25 g was taken aseptically and placed in a sterile stomacher bag with 225 ml of peptone water (Scharlau Chemie, Spain). After 2 min in a stomacher blender (Stomacher 400, Seward Medical, United Kingdom), appropriate decimal dilutions were spread-plated (100 µl) onto Plate Count Agar (PCA) (Scharlau Chemie, Spain) for total viable counts and incubated at 37 °C for 24 h. The results were expressed as logarithms of colony forming units per gram of sample (log CFU/g). Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.8 pH measurement

The pH of patties (10 g homogenised in 50 ml distilled water) was determined using an Orion Model 520A pH metre (AGB Scientific Ltd) throughout the storage period. Three readings were taken for each sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.9 Lipid oxidation measurement

Lipid oxidation was assessed on the basis of the amount of malondialdehyde formed during storage. Malondialdehyde is the end-product of lipid peroxidation and was evaluated using the TBARS assay with some modifications (Oussalah *et al.*, 2006). A 10 g portion of each meat sample was blended with 50 ml of distilled deionised water and 10 ml of 15% trichloroacetic acid (TCA) in a stomacher blender (Stomacher 400, Seward Medical, England) for 2 min at 260 rpm. The homogenate

was centrifuged at 1500 gravity for 5 min and the supernatant fluid was filtered through a Durapore 0.45 µm HV membrane filter (Millipore). A 2 ml aliquot of 60 mmol/L TBA reagent was added to 8 ml of the clear filtrate and vortexed for 15 s and then heated in a boiling water bath for 10 min to develop a pink colour. After cooling on ice to ambient temperature (~ 20 °C), the absorbance was of the supernatant was measured spectrophotometrically at 532 nm (Milton Roy Spectronic 1201). The concentration of malondialdehyde in analysed samples was calculated on the basis of a standard curve obtained using serial dilutions of 1,1,3,3-tetramethoxypropane solution. The TBARS value was expressed as mg malondialdehyde/kg (mg MDA/kg) of sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.10 Extraction of phytochemicals

Seaweed-patty samples (5 g) were crushed in liquid nitrogen using a mortar and pestle, then extracted as described in section 3.2.

9.2.11 Total phenolic content

The total phenolic content of seaweed samples was measured using the Folin-Ciocalteu method (Taga *et al.*, 1994) as described in section 3.4. The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram fresh weight (fw) (mg GAE/100 g fw). Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.12 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as described in section 3.7. Results were expressed as % reduction in DPPH radical scavenging activity. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.13 Texture evaluation

Shear tests were performed as described in section 3.9. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.14 Colour measurement

Colour analysis was carried out as described in section 3.10. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

9.2.15 Sensory characteristics

The consumer acceptance test was conducted in a standardised sensory test room (ISO 9599, 2007). Untrained panelists ($n = 20$) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (25 g) were served on white paper plates with random three-digit numbers and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall acceptability of the product (maximum of 5), where 5 was “like extremely” and 1

was “dislike extremely”. The overall quality (maximum of 25) was computed by combining scores of all five attributes.

9.2.16 Statistical analysis

All experiments were performed in triplicate and replicated twice. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA) as described in section 3.23.

9.3 Results and Discussion

9.3.1 Cooking yield and dietary fibre content of seaweed-patties

Processing loss was the highest in the control sample (40.28% loss) with 59.72% yield remaining after cooking (Fig. 9.2). As seaweed levels were increased cooking losses decreased. The processing losses were 34.80, 34.32, 34.24 and 33.88% for 10, 20, 30 and 40% seaweed concentrations, respectively. This demonstrated that seaweed had a significant effect on cooking loss reductions in seaweed-patties ($P < 0.05$). Cofrades *et al.* (2008) and Fernández-Martín *et al.* (2009) also found that the addition of *H. elongata* improved the water-binding properties of pork meat.

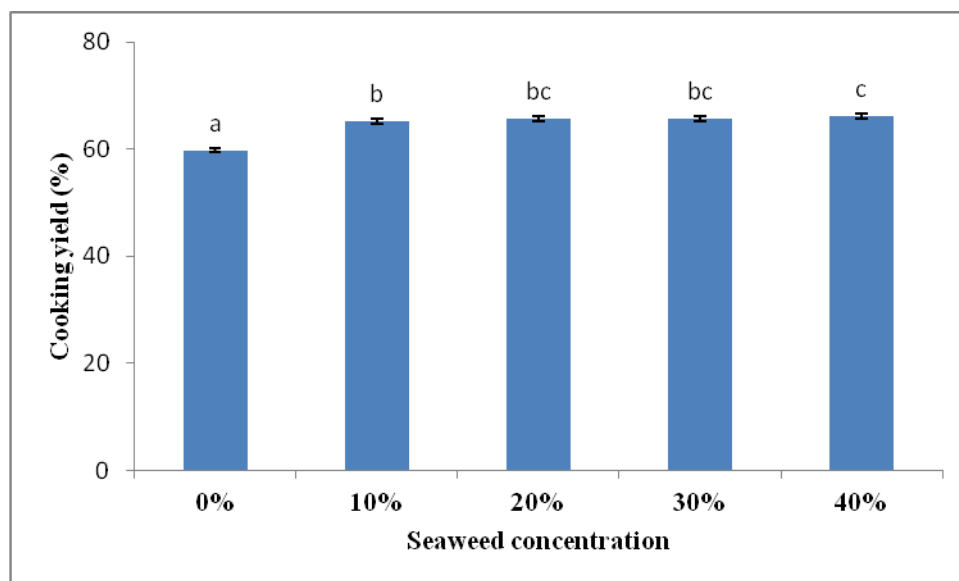


Fig. 9.2 Cooking yield of control and seaweed-patties

Each value is presented as mean \pm SD (n = 6).

Means above each bar with different letters (a-c) differ significantly ($P < 0.05$).

The use of dietary fibre in cooked meat products generally improves hydration properties and fat holding capacity, reducing fat and water loss during cooking and increasing emulsion stability (Thebaudin *et al.*, 1997; Cofrades *et al.*, 2000; Jiménez-Colmenero *et al.*, 2005). The objective of the current study was to incorporate seaweed into beef patties in order to achieve healthier meat products while also producing a product with good sensory attributes such as texture. Seaweeds contain large amounts of dietary fibre and have a high water-holding capacity. The water-holding capacity of seaweeds is closely related to the polysaccharide composition of the dietary fibre fractions, and therefore the gelation process will depend on the type and amount of their polysaccharides (Sánchez-Alonso *et al.*, 2006).

Traditional beef patties are high in fat content (about 14%). Most of this fat is saturated fatty acid (SFA) (about 60% of total fat), while the monounsaturated fatty

acid (MUFA) fraction accounts for about 36% of total fat, and the polyunsaturated fatty acid (PUFA) fraction accounts for about 3% of total fat (Martínez *et al.*, 2011). There are often problems with reduction of fat in finely ground meat products, as it can present a number of difficulties in terms of appearance, flavour and texture. This can cause such products to be less accepted by the consumer (Keeton, 1994; García *et al.*, 2002; Tokusoglu and Ünal, 2003). Manufacturers have introduced several modifications in an attempt to offset the detrimental effects of reducing the fat level. These modifications include the use of non-meat ingredients that could help to convey desirable texture and, more importantly, enhance water-holding capacity (Ako, 1998; Keeton, 1994). In this regard, the incorporation of carbohydrates and fibre have been successful in improving cooking yield, reducing formulation cost and enhancing texture (Keeton, 1994; Jimenez Colmenero, 1996; Mendoza *et al.*, 1998).

In the current study, dietary fibre may have had an important effect on this technological property because it holds water by adsorption and absorption phenomena and some water is also retained outside the fibre matrix (free water) (Sánchez-Zapata *et al.*, 2010). The total dietary fibre content of the control patty and seaweed-patty at a concentration of 40% can be seen in Fig. 9.3.

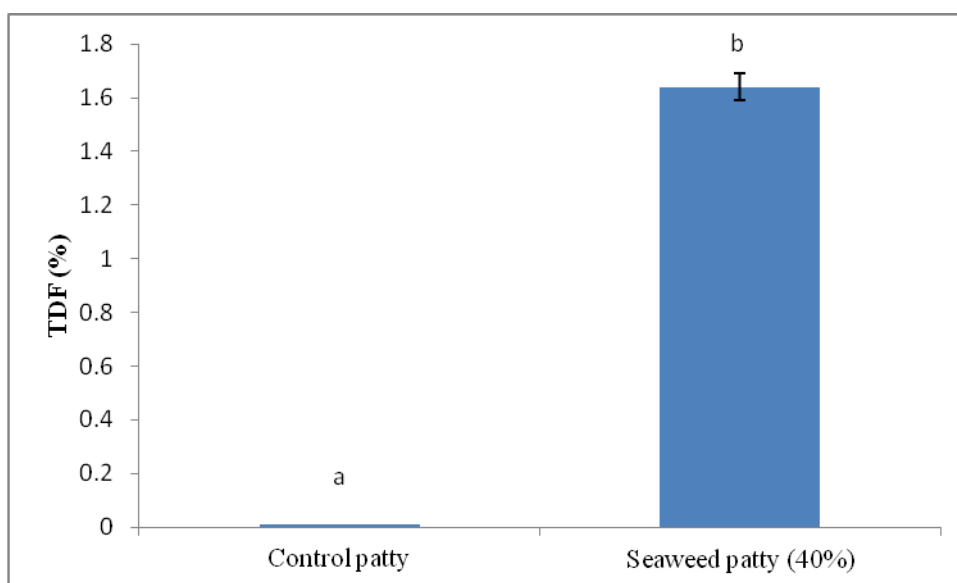


Fig. 9.3 Total dietary fibre content of control and seaweed-patties

Each value is presented as mean \pm SD (n = 3).

Means above each bar with different letters (a-b) differ significantly ($P < 0.05$).

Rehydrated seaweed contained 4.02 g TDF per 100 g fw (4.02%) and when incorporated into patties at 40%, the final product contained 1.64 g TDF per 100 g fw (1.64%). These results are in line with Choi *et al.* (2012) who reported that pork patties with dried *Laminaria japonica* incorporated in the range of 1 to 5% contained 1.23 to 3.14% dietary fibre. Lopez-Lopez *et al.* (2010) reported the TDF in pork patties containing dried seaweed (3%) to be 1.36% in the final product which is also lower than that of the present study; however less seaweed was added as it was in dried form. The recommended daily intake of dietary fibre is > 25 g per day (WHO/FAO, 2003). The addition of fibre to fast food product which is a commonly consumed and low in fibre would help to increase the daily consumption of dietary fibre amongst the population.

9.3.2 Bacterial enumeration and pH of control and seaweed-patties during storage

Microbial growth (log CFU/g) of the vacuum packed seaweed-patties over 30 days of refrigerated storage can be seen in Table 9.1. There was no significant difference in the total viable counts for all patties (control, 10, 20, 30 and 40% seaweed) within the first 14 days of storage as there was no growth of bacteria in any of the samples ($P > 0.05$). There was a significant difference ($P < 0.05$) between the control and the seaweed-patties after 14 days as growth began in the control sample and reached 5.41 log CFU/g by day 30. Generally, the addition of seaweed did not affect the spoilage of patties particularly in samples containing $> 20\%$ seaweed. A low level of growth (1.09 log CFU/g) was seen in seaweed-patties by day 30, and only in patties containing the lowest level of seaweed (10%). This level was however significantly lower than the control samples ($P < 0.05$).

López-López *et al.* (2010) reported that the total viable counts of beef patties and those with added seaweed ranged from 6 - 6.4 log CFU/g. Cofrades *et al.* (2011) also reported that the TVC for restructured poultry steaks with added seaweed were in excess of 6 log CFU/g, however the levels from both these studies are higher than that of the present findings, most likely due to the fact that the patties were uncooked. There are no guidelines specific to total viable counts in minced beef intended to be eaten cooked apart from the requirement for *Salmonella* spp. to be absent in 10 g of sample. Guidelines set out by the Food Safety Authority of Ireland (FSAI) for Enterobacteriaceae numbers on raw meat samples stipulate that three of five samples of raw meat must have counts of < 5 log CFU/g and no more than two of five samples of raw meat can have counts between 5 and 7 log CFU/g. Meat

exceeding these limits is defined as unacceptable. The levels of TVC in the raw patties before cooking in the present study was 2.09 log CFU/g which is well below the FSAI limits and those established by The European Union Commission Regulation (EC No. 2073/2005) on the microbiological criteria for foodstuffs. The pH of the patties (Table 9.1) was also monitored throughout the shelf life as high levels of microorganisms which will result in reductions in pH level (Gómez-López *et al.*, 2007).

Table 9.1 Bacterial enumeration and pH of control and seaweed-patties during storage

Patty	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
Bacterial enumeration (log CFU/g)					
0 days	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
7 days	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
14 days	1.10±0.01by	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
21 days	3.05±0.03cy	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
30 days	5.41±0.02dx	1.09±0.01by	0.00±0.00az	0.00±0.00az	0.00±0.00az
pH					
0 days	6.05±0.03ay	6.04±0.02ay	6.03±0.02az	6.01±0.02az	6.02±0.02az
7 days	6.00±0.01az	6.01±0.02az	6.00±0.03az	6.00±0.02az	6.01±0.03az
14 days	5.96±0.01by	6.00±0.01az	6.00±0.02az	6.00±0.02az	6.00±0.03az
21 days	5.95±0.02by	6.00±0.02az	6.00±0.01az	5.99±0.02az	5.99±0.02az
30 days	5.82±0.01cy	5.99±0.02bz	5.99±0.02bz	6.00±0.03az	6.00±0.03az

Each value is presented as mean ± SD (n = 6, bacterial enumeration; n = 3, pH).

Means within each column with different letters (a – e) differ significantly ($P < 0.05$).

Means within each row with different letters (v – z) differ significantly ($P < 0.05$).

The initial pH values (day 0) of all patty samples were similar ranging from 6.01 to 6.05. These levels are in line with those observed for cooked pork patties with a pH ranging from 6.06 – 6.13 as reported by Choi *et al.* (2012). Significant differences between the control and seaweed-patties were observed after 14 days of storage. The pH values of all seaweed-patties were 6.00, while that of the control was 5.96, which is only slightly lower. By the end of the storage period (30 days) the pH of the seaweed-patties still had not changed significantly ($P > 0.05$) and was in the range of 5.99 – 6.00 while the control had dropped to 5.82. These results are in agreement with those of the bacterial enumeration as the acidity of the control had dropped the most which is most likely due to the increase in bacterial levels as compared to the seaweed-patties.

Vacuum packaging is a preservation packaging method which can greatly enhance the shelf life and overall quality of muscle foods for a long time (Sahoo and Kumar, 2005). In addition, packaging conditions that reduce the amount of oxygen present in the package are known to extend the shelf life of food by inhibiting the growth of aerobic spoilage bacteria (Mendes and Goncalvez, 2008). Therefore the results of the present study demonstrate that the cooked seaweed-patties would have a long shelf life in vacuum packaging as it inhibits microbial spoilage and there also may be an antimicrobial effect due to the addition of seaweed as the control had significantly higher growth than the patties containing seaweed even when stored under vacuum conditions.

9.3.3 Lipid oxidation of control and seaweed-patties during storage

Lipid oxidation generates a series of chemical reactions that can alter the physiochemical parameters, sensorial attributes (odour, colour and flavour) and shelf life in meat and meat products (Liu *et al.*, 1995). TBARS analysis measures the formation of secondary products of lipid oxidation, mainly malondialdehyde, which may contribute off-flavour to oxidized fat (Lee *et al.*, 2011). Lipid oxidation in precooked products remains of concern to the meat industry due to the increased demand for convenience foods. Undesirable flavour in precooked meats, commonly described as warmed-over flavour, rapidly develops in cooked meat products during refrigerated storage (Ahn *et al.*, 2002). Precooked meats are likely to oxidize and produce secondary compounds such as hexanal, pentanal, 2,4-decadienal, 2,3-oxetanedione, and 2-octenal (Trout and Dale, 1990). Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts (Lee *et al.*, 2011).

Table 9.2 shows the effect of different seaweed concentrations on TBARS values of cooked-patties during 30 days of storage. Initial TBARS levels (Day 0) of all samples were similar ranging from 0.18 to 0.20 mg malondialdehyde/kg (mg MDA/kg). TBARS values of all patties containing seaweed were significantly lower ($P < 0.05$) than the control during storage. The levels of TBARS began to increase at 14 days of storage. This indicated that there was some protective effect of the seaweed against lipid oxidation in cooked minced beef, potentially due to the increase in phenolic compounds and DPPH activity as discussed in sections 9.3.4 and 9.3.5. The reduction in lipid oxidation could also be due to the reduction in meat

content in the samples (10 – 40% less meat) which accordingly would have lower levels of fat present in the samples thus reducing potential oxidation.

Table 9.2 Lipid oxidation of control and seaweed-patties during storage (mg malondialdehyde/kg)

Day	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
0	0.19±0.03ax	0.20±0.01ay	0.18±0.02az	0.19±0.01ax	0.19±0.04ax
7	0.45±0.05bv	0.25±0.03bw	0.27±0.03bx	0.22±0.01by	0.24±0.06bz
14	0.77±0.05cv	0.40±0.06cw	0.38±0.01cx	0.39±0.03cy	0.45±0.06cz
21	0.89±0.04dv	0.61±0.05dw	0.55±0.05dx	0.57±0.04dy	0.56±0.02dz
30	1.12±0.02ew	0.69±0.02ex	0.69±0.06ex	0.66±0.02ey	0.61±0.02ez

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a – e) differ significantly ($P < 0.05$).

Means within each row with different letters (v – z) differ significantly ($P < 0.05$).

The differences in TBARS values of seaweed-patties ranged from 0.18 – 0.69 mg MDA/kg from the beginning to end of storage. Therefore, the extent of this lipid oxidation during refrigerated storage may be considered relatively low according to Bhattacharya *et al.* (1988), Rojas and Brewer (2007) and López-López *et al.* (2010). The results of the present study are in agreement with López-López *et al.* (2010) who reported that the TBARS values of seaweed-patties ranged from 0.27 – 0.87 mg MDA/kg during frozen storage.

These authors also reported that due to the level of polyphenols which remained from seaweed incorporated in the meat coupled with the relatively low TBARS values, it could be assumed that little antioxidant activity was required from the seaweed due to the low level of lipid oxidation in the products. This is of interest from a nutritional point of view given the potential beneficial effects of these

bioactive compounds in the product which when consumed have an impact on the body redox status and the antioxidant polyphenols on human health through their potential role in the prevention of chronic diseases (Bocanegra *et al.*, 2009).

Another benefit of the product is that it can be packaged in vacuum conditions which can prevent lipid oxidation. Vacuum packaging and storage under chilled conditions have proved to be very effective for extending the shelf-life of various perishable foods, and so facilitating long-term storage and intercontinental transport of such foods (Pennacchia *et al.*, 2011).

9.3.4 Total phenolic content of control and seaweed-patties during storage

The total phenolic content (TPC) of the seaweed-patties over the 30 days of storage is shown in Fig. 9.4. Phenolic compounds exist as various structures, have different molecular weights and are related to the innate flavour of food. They contain a phenolic hydroxyl group, which has an antioxidative effect through interactions with the phenol ring and has a resonance stabilization effect (Shahidi and Wanasundara, 1992). Differences in the TPC of all samples were significant ($P < 0.05$). The control sample contained no detectable phenols at tested levels, while the TPC increased significantly ($P < 0.05$) with increasing seaweed concentrations (10 – 40%). The TPC ranged from 7.05 – 28.11 mg GAE/100 g fw and by day 30 these levels were 6.42 – 24.21 mg GAE/100 g fw.

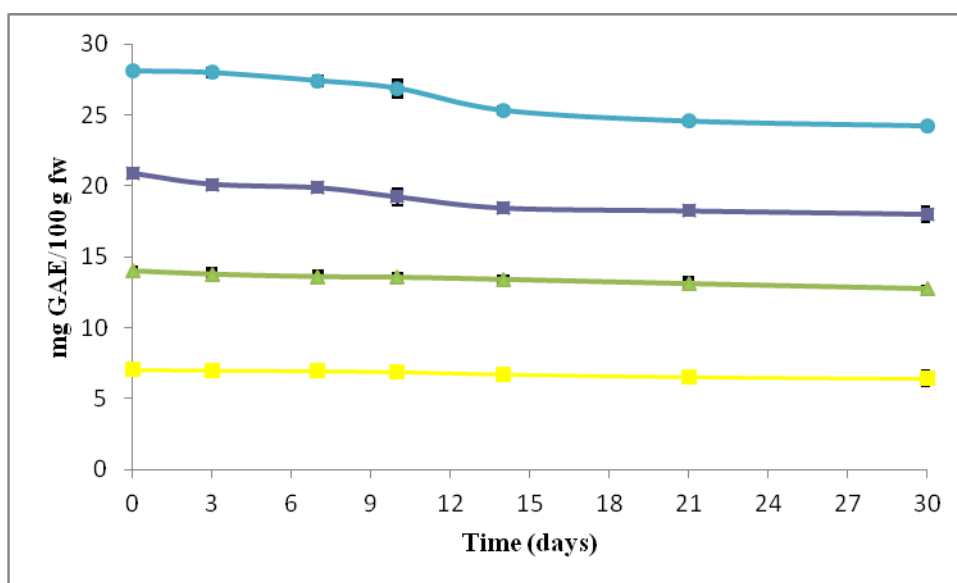


Fig. 9.4 Total phenolic content of control and seaweed-patties during storage (□: 10%; △: 20%; □: 30%; ○: 40% seaweed)

9.3.5 DPPH radical scavenging activity of control and seaweed-patties during storage

DPPH is a free radical compound widely used to determine the free radical-scavenging ability of various samples (Amarowicz *et al.*, 2004). The DPPH radical scavenging activity of the patties over 30 days of storage is presented in Fig. 9.5. The control sample contained no detectable phenols at tested levels. The initial levels of DPPH scavenging activity in all seaweed-patty samples were significantly different ($P < 0.05$) and ranged from 30.23 – 52.34%. Throughout the storage period the DPPH activity reduced significantly for each of the seaweed-patty samples ($P < 0.05$). By day 30 levels were in the range of 26.65 – 40.69% for the different concentrations of seaweeds. Such an improved initial antioxidant capacity in the seaweed-patties clearly points to an enhancement in the quality of the meat products when the seaweeds were incorporated, as it could also improve the antioxidant stability of the meat products during processing and storage (Lee *et al.*, 2006a; Lee *et*

al., 2006b; Valencia *et al.*, 2007). The increased levels of radical scavenging activity could also have led to reductions in the lipid oxidation during storage.

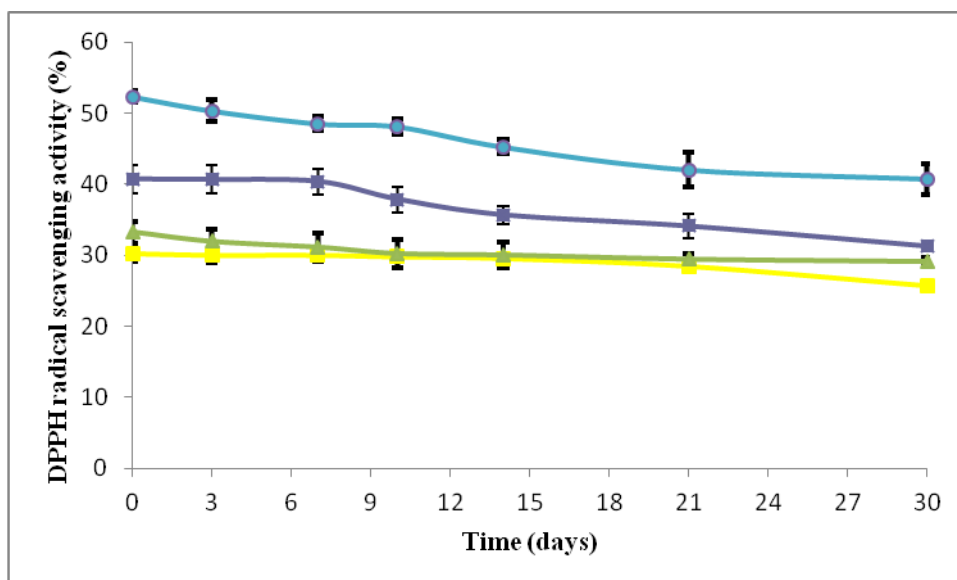


Fig. 9.5 DPPH radical scavenging activity of control and seaweed-patties during storage (□: 10%; △: 20%; ▢: 30%; ○: 40% seaweed)

9.3.6 Texture of control and seaweed-patties during storage

The firmness/tenderness of the texture of the patty samples throughout storage is shown in Table 9.3. The initial tenderness of each of the patties (control, 10, 20, 30 and 40% seaweed) were all significantly different ($P < 0.05$) ranging from 17.50 – 19.06 N/mm. As seaweed levels increase, the patties become more tender. An addition of 40% seaweed represented a 46.98% difference in tenderness levels compared to that of the control. Dietary fibres from different sources have been studied for formulation of different meat products, with a view, among other things, to improve texture. It has generally been found that addition of such fibres to meat augmented firmness (Cofrades *et al.*, 2008; Fernández-Martín *et al.*, 2009; Sánchez-Zapata *et al.*, 2010). However, while some authors have observed increases in

firmness with the addition of fibres to meat, others have found no difference or the production of more tender products (Chun *et al.*, 1999; Cofrades *et al.*, 2000; Jiménez-Colmenero *et al.*, 2005; Selgas *et al.*, 2005). López-López (2010) also reported that beef patties containing seaweed were more tender than the control. The effect of seaweed addition on the tenderness of the patties was due to the role played by fibre as the protein content of both control and seaweed-patties was similar. The tenderness of all of the samples in the present study increased (became firmer) throughout storage ($P < 0.05$). By the end of the storage period (30 days) the tenderness of the samples ranged from 21.33 – 40.23 N/mm, with the firmest being the control and most tender in patties containing the highest levels of seaweed (40%). The patties were more tender as seaweed concentrations increased, therefore patties containing 40% seaweed were most tender throughout the shelf life. The texture of the control samples was almost double that of those containing 40% seaweed. This is due to the reduction in cooking losses as the seaweed-patties retained more water and thus keeping the product more moist and tender throughout shelf life. In order to determine if this difference was acceptable amongst consumers, sensory analysis was carried out as discussed in section 9.3.8.

Table 9.3 Firmness/tenderness of control and seaweed-patties during storage (N/mm)

Day	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
0	18.06±1.68av	19.06±1.16aw	17.63±1.35ax	17.50±1.10ay	17.77±1.34az
7	25.33±2.31bv	21.25±1.55bw	19.82±1.94bx	18.88±2.30by	18.54±1.25bz
14	32.76±3.30cv	25.11±3.32cw	23.42±2.30cx	22.38±2.38cy	20.11±3.33cz
21	38.22±1.98dv	26.77±2.33dw	24.02±1.34dx	22.78±2.87dy	20.87±2.10dz
30	40.23±1.76ev	28.44±3.54ew	24.54±2.04ex	23.98±2.12ey	21.33±3.45ez

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a – e) differ significantly ($P < 0.05$).

Means within each row with different letters (v – z) differ significantly ($P < 0.05$).

9.3.7 Colour of control and seaweed-patties during storage

Colour was evaluated in order to detect the tendencies for seaweed addition to cause changes in the beef-patties, given that colour is one of the main parameters determining consumer acceptance of a product (Cofrades *et al.*, 2008). Seaweed addition had an immediate effect on colour parameters of patties in comparison to the control (Table 9.3). At the initial stage (day 0), the L^* values of the patty samples with seaweed incorporated were higher than that of the control (colour was lighter). Seaweed concentrations (10 – 40%) also had a significant effect on the L^* values as the patties became lighter in colour with increasing seaweed levels ($P < 0.05$). It has been reported that usually in meat products, the higher the moisture content, the higher the lightness (L^*) value (Pérez-Alvarez *et al.*, 1999; Alesón-Carbonell *et al.*, 2005; Fernández-López *et al.*, 2008). The higher L^* values could therefore also be due to the high moisture content of the seaweed and the moisture retention upon cooking as compared to the control.

The a^* values of the samples were significantly different (day 0) as compared to the control ($P < 0.05$), with values ranging from 7.05 (10% seaweed) to 8.39 (control). This parameter is a measure of the redness/greenness of a sample with lower a^* readings containing more green pigments. This would explain the reduction in a^* values as compared to the control as blanched *H. elongata* is bright green in colour. The initial b^* values (day 0) were significantly ($P < 0.05$) higher than the control patties containing no seaweed. This parameter is a measure of the yellowness/redness of the samples and the higher b^* values of the seaweed-patties indicate an increase in yellow colour.

With respect to colour during storage; L^* values changed significantly for all samples ($P < 0.05$). The L^* values decreased by day 30, indicating a slight darkening of the samples, with the exception of patties with 30 and 40% seaweed which became slightly lighter in colour. There was a significant increase in a^* values for all samples (except 20 and 30% seaweed-patties) by day 30, which indicated that the redness of the samples increased slightly, therefore there was a reduction of the green colour of the blanched seaweed. There was also a significant increase in b^* values for all samples (except 10 and 20% seaweed-patties) by day 30. This indicates that there was a reduction of the yellowness of the samples.

Although there were differences in the colour values throughout the storage period, most of the colour parameters of the patty samples were basically steady (slightly changed) which was also reported by Shan *et al.* (2009) who studied the effects of adding spice and herb extracts to raw pork. Although the addition of seaweed changed the colour of the patties as compared to the control, this is in line with meat

colour changes upon the addition of spice and herbs which are traditionally added to meats. In order to determine the acceptability of the colour, this was taken into account in the sensory analysis (section 9.3.8).

Table 9.3 Colour of control and seaweed-patties during storage (Hunter L*, a*, b*)

Coordinate	Day	Control (0% seaweed)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
L*	0	36.63±0.22aw	39.06±0.08ax	39.08±0.16ax	40.12±0.03ay	40.25±0.11az
	7	35.89±0.56bv	37.08±1.23bw	37.89±0.23bx	40.15±0.80by	41.58±1.12bz
	14	34.63±0.11cv	37.99±0.47cw	37.66±0.29cx	41.25±0.88cy	40.99±0.87cz
	21	34.39±1.18dv	37.39±0.85dw	37.56±0.10dx	41.72±1.02dy	40.12±0.17dz
	30	35.49±1.12ev	37.45±0.52ew	38.12±0.23ex	41.56±1.6ey	40.32±1.07ez
a*	0	8.39±0.04av	7.05±0.33aw	7.96±0.24ax	7.99±0.12ay	8.32±0.09az
	7	8.73±0.09bv	7.12±0.44bw	8.23±0.20bx	8.01±0.39by	8.33±0.56az
	14	9.70±0.56cv	6.96±0.56cw	7.99±0.34cx	8.22±0.23cy	8.87±0.41bz
	21	9.37±0.45dv	6.98±0.25dw	7.58±0.03dx	7.97±0.25dy	8.12±0.57cz
	30	8.91±0.78ev	7.88±0.23ew	7.77±0.87ex	7.87±0.33ey	8.56±0.41dz
b*	0	14.22±0.12av	16.67±0.11aw	16.00±0.02ax	16.54±0.14ay	16.66±0.13az
	7	15.51±0.54bw	16.69±0.14ax	15.97±0.25by	16.99±0.10bz	16.67±0.66az
	14	15.82±0.12cv	16.61±0.45bw	16.04±0.30cx	17.11±0.03cy	17.25±0.49bz
	21	15.21±0.13dv	16.55±0.78cw	15.97±0.24dx	17.10±0.65cy	17.32±0.23cz
	30	15.74±0.45ev	16.56±1.10dw	15.93±0.55ex	16.67±0.70dy	17.22±0.87dz

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a – e) differ significantly ($P < 0.05$).

Means within each row with different letters (v – z) differ significantly ($P < 0.05$).

9.3.8 Sensory analysis

Table 9.5 summarises the sensory scores for aroma, appearance, texture, taste and overall acceptability of control and seaweed-patties. The samples tested by the consumers were the control (with no added seaweed), a mid-range seaweed-patties (20% seaweed) and patties with 40% added seaweed which would have the maximum level of antioxidants and TDF. Aroma, appearance, texture and taste of the seaweed-patties were found to be significantly different to the control ($P < 0.05$). The sensory scores for aroma ranged from 4.23 (20% seaweed) to 4.61 (control). Although there was a significant difference, the results were all within an average score of 4 which indicted that there wasnt a major difference in the detection of a seaweed aroma as compared to the control. The fact that a strong seaweed aroma was not detected could potentially be due to the fact that the seaweeds were blanched before being added to the meat.

The sensory score for appearance ranged from 4.23 to 4.84, with the score reducing with increasing seaweed concentration. This showed that the patties without the incorporation of seaweed were more visually appealing to consumers, however the mean score for all samples was still above 4, which is a positive result. The scores for texture were significantly higher with increased levels of seaweed ($P < 0.05$). This is a positive result as consumers detected that seaweed improved the texture and possible mouthfeel of the patties which was one of the objectives of the study. The addition of blanched seaweeds over dried seaweeds in the present study offers exploitation of the gelling properties of the seaweeds. This would also contribute to the technological properties of the seaweed such as reducing cooking losses.

The seaweed-patties also had a significantly higher score for taste than the control with 20% seaweed-patties ranking the highest ($P < 0.05$). The 40% seaweed-patty ranked highest in the overall acceptability score ($P < 0.05$) with the control receiving the lowest score. This indicated that incorporation of seaweed in the patties had a positive effect on the overall acceptability of the product. The results of the present study are promising particularly when compared to those reported in literature. Piñero *et al.* (2008) found that the taste scores for beef patties with added oat fibre to be lower than the control. Cofrades *et al.* (2011) reported that while all restructured poultry steaks with added *H. elongata* were judged acceptable by a sensory panel, the control received a higher score for overall acceptability than those containing seaweed while Choi *et al.* (2012) stated that sensory evaluations indicated that the greatest overall acceptability in pork-patties was also attained in samples containing seaweed.

Table 9.5 Mean scores for aroma, appearance, texture and taste of the control and seaweed-patties

Patty	Sensory attributes				
	Aroma	Appearance	Texture	Taste	Overall acceptability
Control	4.61±0.66a	4.84±0.37a	3.00±0.95a	3.76±0.61a	3.75±1.64a
20% seaweed	4.23±0.83b	4.30±0.48b	3.07±0.44b	4.23±0.83b	4.09±0.88b
40% seaweed	4.38±0.77c	4.23±0.59c	3.69±0.49c	4.15±0.80c	4.25±0.78c

Each value is presented as mean ± SD (n = 20).

Means within each column with different letters differ significantly ($P < 0.05$).

9.4 Conclusion

The addition of *H. elongata* to meat products in the development of functional foods opens up new potential for seaweed utilisation. Incorporating such seaweeds is of

interest from a technological and functional point of view. The seaweed had a positive effect on the cooking yield of the patties due to hydrocolloids in the seaweed which reduce cooking losses. Total dietary fibre, polyphenolic content and antioxidant activity were increased due to the incorporation of seaweed. Storage life was enhanced in samples containing seaweed as compared to the control and lipid oxidation was also greatly reduced due to the levels of phytochemicals present in the seaweed. The seaweed also had a positive effect on the texture of the patties as they were more tender than the control which was also confirmed in the sensory analysis study. The seaweed-patties were found overall to be acceptable by a sensory panel, particularly in terms of texture. Such positive attributes of the seaweed could therefore be exploited for the development of new functional foods and would offer the meat industry a means to improve the “image” of such commonly consumed fast foods.

CHAPTER 10

GENERAL CONCLUSIONS

A summary of the main conclusions arising from the work, including suggestions for further research

10.1 General conclusions

Edible Irish seaweeds; *L. digitata*, *L. saccharina*, *H. elongata*, *P. palmata*, *C. crispus* and *E. spirulina*, successfully displayed both antioxidant and antimicrobial activities. Brown seaweed, *H. elongata*, had the highest antimicrobial and antioxidant content of the six studied species. The extraction of antimicrobials from the different species of seaweeds was solvent dependent and that methanol was a good solvent for antimicrobial extraction from brown seaweeds, whereas acetone was better for red and green species. The antioxidant content of the seaweeds did not vary significantly with different polar solvents.

Seaweed processing methodologies significantly influenced the concentrations of phytochemicals (antioxidant and antimicrobials) in *H. elongata*. As some processing is required to make *H. elongata* edible, and since heat treatment can lead to a loss of phytochemical properties, a compromise must be reached between palatability and nutrition. A combination of drying followed by boiling reduced processing time and led to less leaching of phytochemicals. Heat processing significantly affected the antimicrobial activity of extracts from *H. elongata* with all processing treatments significantly reducing the antimicrobial activity. Therefore, extracts from fresh *H. elongata* had the highest antimicrobial activity against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* with good inhibition as low as 4.16 mg/ml extract.

The first stage of the work, described in chapters 4 and 5, allowed for a screening of the bioactivity levels of each seaweed and the effect which common processing procedures have upon them. Dehydration and rehydration kinetics of seaweeds were the studied in more depth in the second stage of the project. Dehydration kinetics

were accurately predicted using the empirical models of Newton, Logarithmic and Henderson-Pabis. Dehydration reduced the phytochemical constituents in the seaweed, a reduction of 29% in the TPC and 30% in the TFC was observed when *H. elongata* was dried at 40 °C for 24 h. However, when the seaweed was dried up to 50% moisture content, a significant increase of 41% in the TPC was observed. Seaweed dried at 40 °C for 24 h had a 12.3% increase in TTC and reduction in DPPH scavenging was minimal under the same conditions with only a 4.5% loss of activity. This showed that the semi-dried form of seaweeds is even more nutritious and rich in bioactives than the raw state.

Once optimisation of the dehydration procedure was established, rehydration of the dried seaweed under a range of temperatures was investigated. Rehydration kinetics of *H. elongata* were accurately predicted using the empirical models of Weibull, Peleg's, first-order and exponential association. Rehydration kinetics increased linearly with the time and temperature applied until it reached equilibrium, however there were significant losses in phytochemical content during the rehydration procedure. Moisture equilibrium was achieved most quickly at 100 °C after 40 min. Under these rehydration conditions, losses of up to 93% were seen in the phytochemicals, as compared to dried seaweed. Colour and texture were also significantly changed during the dehydration and rehydration procedures and modelling of texture kinetics were successfully fitted to zero- and first-order models. The dehydration and rehydration processes therefore need to be controlled in order to minimise phytochemical losses while at the same time processing the product to an edible texture.

A bakery product namely breadsticks were developed in this research employing Response Surface Methodology (RSM) in order to determine levels of dried seaweed and white/wholemeal flour combinations which would maximise phytochemical and fibre levels. From the response surface plots, seaweed concentration was found to have the most significant effect on phytochemical content of the breadsticks. The high coefficients of determination of the variables at a 95% confidence level indicated that second order polynomial models could be employed to predict critical phytochemical parameters of breadsticks containing *H. elongata* along with texture and colour. The addition of seaweeds to breadsticks offer products with higher levels of dietary fibre and phytochemicals as compared to control samples and with an appealing colour and texture as judged by a sensory panel.

A second seaweed based product was developed, based on the incorporation of 40% rehydrated *H. elongata* in beef-patties in order to improve the “image” of a fast food product which is generally low in fibre. Total dietary fibre, polyphenolic content and antioxidant activity were increased due to the incorporation of seaweed. Storage life was enhanced in samples containing seaweed as compared to the control and lipid oxidation was also greatly reduced. The incorporation of seaweeds in meat patties also had a positive effect on the texture of the patties as they were more tender than the control as confirmed by the sensory panel. Overall the seaweed-patties were found acceptable by a sensory panel, particularly in terms of texture and received higher scores for texture and overall acceptability as compared to the control upon incorporation of 40% seaweed.

10.2 Suggestions for future work

The functional food products developed in the work could potentially be marketed as novel food products which are both convenient and healthy. The processing steps carried out on the seaweed in the present study were conducted under laboratory conditions. Therefore in order to produce larger volumes of sample to support such a functional food industry, appropriate scaling up of the dehydration and rehydration procedures would be required. As traditional sun drying under atmospheric conditions is time consuming and managed unscientifically, such as monitoring dryness by touch, employing driers within the Irish seaweed industry would offer a science-based approach which could capitalise on the nutritional content of seaweed.

Currently most seaweeds are washed, dried and packaged with little done to increase consumer appeal. Many consumers would also be unfamiliar with how to use dried seaweeds therefore incorporation of pre-optimised seaweeds into commonly consumed products would be an interesting application. Therefore the dried and rehydrated seaweeds developed in the present study, could be used as functional ingredients to be added to other food products thus supporting the seaweed industry by tapping into the undisputed wealth of such an abundant natural resource.

There is potential to exploit the extracts in order to enhance food safety, research could be conducted to explore the opportunities of adding the extracts into food products in order to extend their shelf life. Adding the extracts to perishable foods such as minced meat could potentially prevent lipid oxidation and delay microbial growth thus extending the shelf life. The extracts could also be applied in foods as a

potential source of added phytochemicals. This could be a benefit in products where antioxidants are generally low such as meat or breads.

Clinical trials could be carried out to determine the health benefits of consumption of the functional foods produced on the diet. These functional foods could potentially be designed to combat obesity as the seaweed has an effect on satiety as it bulks food and has a filling effect while also being low in fat and calories.

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Cox, S., Abu-Ghannam, N. and Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, 17, 205-220.

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Cox, S. and Abu-Ghannam, N. (2013). Enhancement of the phytochemical and fibre content of beef-patties with *Himanthalia elongata* seaweed. *International Journal of Food Science and Technology*, in press.

Conference presentations

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